Genetic and clinical aspects of inherited syndromes associated with adenomatous polyposis

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To my Parents

Javid & Mina

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CHAPTER 1

General Introduction

GENERAL INTRODUCTION

Hereditary colorectal cancer (CRC) syndromes account for 5-15% of colorectal cancers. Hereditary CRCs are often divided into two major categories: Hereditary polyposis syndromes and non-polyposis syndromes.

Lynch syndrome, an autosomal dominant non-polyposis syndrome, is the most common form of hereditary CRC, responsible for 3-5% of all CRC cases. It is characterized by high risks of developing colorectal and endometrial cancer as well as cancer of urinary tract, stomach, small intestine and other cancers.[1]

Hereditary polyposis syndromes are a group of syndromes characterized by the development of multiple colorectal polyps and/or histopathologically specific polyps and a high risk of developing colorectal cancer at an early age.[2] Familial adenomatous polyposis (FAP) is the most common polyposis syndrome responsible for 1% of all CRC cases.[3] Other less common polyposis syndromes include: MUTYH-associated polyposis (MAP), Juvenile polyposis syndrome (JPS), Peutz-Jeghers syndrome (PJS), PTEN hamartoma tumor syndrome and Constitutional mismatch-repair deficiency syndrome (CMMRD). More recently, next-generation sequencing has identified novel genetic causes of hereditary polyposis syndromes such as polymerase proofreading-associated polyposis (POLD1- and POLE-associated polyposis) and NTHL1- associated tumor syndrome.[2]

PART I: APC-ASSOCIATED POLYPOSIS/FAMILIAL ADENOMATOUS POLYPOSIS (FAP) & MUTYH-ASSOCIATED POLYPOSIS (MAP)

A. APC-ASSOCIATED POLYPOSIS / FAMILIAL ADENOMATOUS POLYPOSIS (FAP)

APC-associated polyposis is an autosomal dominant cancer predisposition syndrome caused by a germline pathogenic variant in the APC gene. About one third of cases are due to *de novo* germline APC. It is estimated that 20% of the *de novo* cases of FAP have somatic mosaicism.[4,5]

Colonic manifestations of FAP include the development of hundreds to thousands of adenomatous polyps. Approximately 50% of FAP patients develop adenomas by age 15 and 95% by age 35.[6] CRC develops a decade after the appearance of polyps and without intervention, the risk is almost 100% by the

age of 50.[7] Attenuated FAP (AFAP) is observed in 8-10% of cases and is characterized by a milder colonic phenotype with <100 adenomatous polyps developing at a more advanced age.[2] Besides the colonic phenotype, patients present with other manifestations including duodenal adenoma and cancer, desmoid tumor and extraintestinal malignancies.

The discovery of the APC pathogenic variants as the cause of FAP, have enabled the identification of FAP in pre-symptomatic individuals. The implementation of surveillance programs and preventive surgical intervention improved the life-expectance of these patients considerably. Due to colorectal surveillance, a shift was observed in the causes of death with less deaths due to CRC and more deaths due to desmoid tumors and duodenal cancer.[8–10]

Genetics

APC is a tumor suppresser gene located on chromosome 5q21-22. The gene consists of 15 exons which encodes a 2843-amino acid protein.[11,12] The APC protein is a component of the Wnt/β-catenin signaling pathway. APC complexes with glycogen synthase kinase-3 (GSK3) and axin and negatively regulates β-catenin by promoting the phosphorylation and ubiquitination. In APC mutation, accumulated β-catenin interacts with T-cell factor (TCF) transcription factor which leads to transcriptional activation of specific genes involved in proliferation, differentiation, migration, apoptosis, and cell-cycle progression.[13,14]

Genotype phenotype studies

Several studies have reported an association between the location of mutation on the APC gene and the severity of colonic disease reflected by the number of polyps and age of onset.[15,16] Correlations between attenuated FAP (AFAP) and mutations before codon 157, after codon 1595 and in the alternatively spliced region of exon 9 are described. Severe polyposis (>1000 adenomas) is correlated with mutations between codons 1250 and 1464 and mutations in the remainder of the APC gene are responsible for an intermediate colonic phenotype.[15]

In addition, attempts have been made to correlate extracolonic features with the site of APC mutation. An association between congenital hypertrophy of the retinal pigment epithelium (CHRPE) and desmoid tumors was observed with mutations between codons 311 and 1444 and after codon 1444, respectively.[15]

Modifier genes in FAP

Phenotypic variability within FAP families with the same APC mutation suggests that besides genotype, other factors play a role in severity of polyposis and risk of CRC. Several modifier genes have been investigated and some genes were found to influence disease severity in FAP.[17–20]

The phenotypic variability among different inbred strains of the Apc (Min) mouse model also highlights the importance of modifier alleles in FAP and several modifiers of Min (Mom) loci associated with the Apc (Min) mutation have been identified to date.[21–23] These modifiers such as Mom1 (modifier of Min 1) and Mom5 which alter tumor phenotypes have also been studied extensively.[24–26]

In the general population, environmental and genetic factors influence risk of CRC.[27] In addition, several single nucleotide polymorphisms (SNPs) identified by genome-wide association studies (GWAS) show an association with sporadic CRC.[28] **Chapter 2** describes a research project that studies the influence of CRC-associated SNPs on disease phenotype in patients with a germline pathogenic variant in APC.

Extracolonic manifestations

Besides colorectal polyposis, many extracolonic manifestations, including benign and malignant features, are observed in FAP patients. Benign extracolonic manifestations include congenital hypertrophy of retinal pigment epithelium (CHRPE), fundic gland polyps, epidermal cysts, dental anomalies and adrenal masses. Duodenal adenoma is found in 80-90% of patients and the risk of duodenal cancer is 5-15%. Other less frequently observed extracolonic neoplasms include cancers of thyroid, brain, stomach, pancreas, liver and desmoid tumors.[20,29]

Substantial decrease in mortality due to CRC has increased the life expectancy of FAP patients. As a consequence, more patients die from other causes including extra-colonic cancers. [6,30,31] The risk of extracolonic cancers such as thyroid, liver and pancreatic cancer in FAP reported in the literature varies widely which led to different opinions regarding the need of surveillance. In **chapter 3**, the occurrence of extracolonic malignancies and the causes of death in a large series of adenomatous polyposis coli (APC) mutation carriers is evaluated and the option of additional surveillance for these cancers is discussed.

B. MUTYH-ASSOCIATED POLYPOSIS

MUTYH-associated polyposis (MAP) is an autosomal recessive cancer predisposing syndrome found in 10-20% of patients with polyposis. The MUTYH gene is located at chromosome 1 locus, lp34 and has 16 exons. MUTYH is a base-excision repair gene involved in oxidative DNA damage repair.

Patients with MAP have a variable colonic phenotype but usually present with mild polyposis in the third and fourth decade of life. Hyperplastic polyps and sessile serrated adenomas also occur in MAP. The risk of CRC in MAP is significantly increased (75.4% and 71.7% for males and females, respectively).[32-36]

Gastric and duodenal polyps are found in 11% and 17% of MAP patients, respectively, and the life time risk of duodenal cancer is estimated around 4%. In addition, an increased risk has been reported for various cancers including ovarian, bladder, breast and endometrial cancer. Sebaceous gland tumors (adenomas, epitheliomas and carcinomas) are found in about 2% of MAP patients. In addition, benign adrenal lesions, thyroid nodules, jawbone cysts, and congenital hypertrophy of the retinal pigment epithelium have been reported.[35–37]

Recently, a study revealed a high frequency of Barrett's esophagus (BE) in patients with FAP.[38] BE is characterized by replacement of the normal squamous epithelium with columnar epithelium and is the only known precursor lesion of esophageal adenocarcinomas which is associated with gastro-esophageal reflux disease (GERD) and an increased incidence of colorectal adenomas.[39–42] It is

unknown whether BE is also more frequent in MAP patients. In **chapter 4**, we therefore studied the prevalence of BE in a large cohort of patients with MUTYH-associated polyposis and APC-associated adenomatous polyposis.

PART II: CONSTITUTIONAL MISMATCH REPAIR DEFICIENCY (CMMRD)

Lynch Syndrome is an autosomal dominant disorder caused by a defect in one of the DNA mismatch repair genes, MLH1, MSH2, MSH6 and PSM2.[43] Homozygous and compound heterozygous pathogenic variants in these genes result in a rare cancer predisposition syndrome called constitutional mismatch repair deficiency (CMMRD).

Tumor spectrum

Patients with CMMRD develop hematological malignancies and brain tumors in the first decade of life. Malignancies of the digestive tract including CRC and small bowel tumors as well as lynch syndrome tumors such as endometrial cancers and urinary tract malignancies are diagnosed in the second and third decade of life. Non-malignant lesions may include café-au-lait macules (CALMs), mild immunoglobulin deficiencies and congenital malformations.[44,45]

Surveillance programs

Surveillance protocols have been established which has resulted in improved care for patients with various hereditary CRC syndromes.[46–48] Also, follow-up of a family with CMMRD over 10 years has proved to be effective and resulted in diagnosis of fifteen tumors.[49] However, until recently, no formal guidelines for surveillance and management of CMMRD, were available.

In 1968, a set of criteria was proposed by the WHO that should be met prior to the implementation of screening programs. These criteria include: (a) cancer should be a common problem in the target group of surveillance; (b) the natural course of the cancers should be known; (c) screening tests should be available with high sensitivity and specificity and the tests should be acceptable for the patients; (d) an effective treatment should be available after detection of the tumor; (e) there should be evidence that screening leads to diagnosis of cancer

at an early stage and to improvement of prognosis; (f) finally, the surveillance protocol should be cost-effective.[50]

In **chapter 5**, we reviewed the literature and collected data on the tumor spectrum, the clinical presentation and the natural course of the most common cancers in CMMRD including brain tumors, digestive tract cancers and hematological cancers. We then evaluated whether surveillance for various cancers complied with the above-mentioned criteria. Based on this review and discussions among the members of the European collaborative group (Care for CMMRD (C4CMMRD)), we developed guidelines for surveillance and management of patients with CMMRD.

The C4CMMRD group established a European CMMRD patient database at the Gustave Roussy Cancer Campus in Villejuif, France. The aim of this registry was collecting prospective data to evaluate the outcome and effectiveness of the surveillance protocol in CMMRD patients. In **chapter 6,** the results of surveillance of CMMRD patients that participated in the program are reported.

AIMS OF THIS THESIS

- To assess whether known CRC-associated SNPs influence the disease phenotype in patients with a germline pathogenic variant in APC.
- To investigate the occurrence of extracolonic malignancies and benign tumors in a large series of patients with FAP with a proven APC pathogenic variant known from the Dutch polyposis registry and to evaluate whether these extracolonic malignancies are an important cause of death.
- To assess the prevalence of Barrett's esophagus and esophageal adenocarcinomas by reviewing the endoscopy reports in a large cohort of patients with FAP and MAP.
- To develop a surveillance program to detect the most common cancers at an early or premalignant stage in patients with CMMRD
- To assess the effectiveness of the C4CMMRD surveillance program and discuss possible improvements of the protocol.

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PART I

APC-ASSOCIATED POLYPOSIS/
FAMILIAL ADENOMATOUS POLYPOSIS (FAP)
&
MUTYH-ASSOCIATED POLYPOSIS (MAP)

CHAPTER 2

Colorectal Cancer Risk Variants at 8q23.3 and 11q23.1 are Associated with Disease Phenotype in APC Mutation Carriers

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ABSTRACT

Familial adenomatous polyposis (FAP) is a dominantly inherited syndrome caused by germline mutations in the *APC* gene and characterized by the development of multiple colorectal adenomas and a high risk of developing colorectal cancer (CRC). The severity of polyposis is correlated with the site of the *APC* mutation. However, there is also phenotypic variability within families with the same underlying *APC* mutation, suggesting that additional factors influence the severity of polyposis. Genome-wide association studies identified several single nucleotide polymorphisms (SNPs) that are associated with CRC. We assessed whether these SNPs are associated with polyp multiplicity in proven *APC* mutation carriers. Sixteen CRC-associated SNPs were analysed in a cohort of 419 *APC* germline mutation carriers from 182 families. Clinical data were retrieved from the Dutch Polyposis Registry. Allele frequencies of the SNPs were compared for patients with <100 colorectal adenomas versus patients with ≥100 adenomas, using generalized estimating equations with the *APC* genotype as a covariate. We found a trend of association of two of the tested SNPs with the ≥100 adenoma phenotype: the C alleles of rs16892766 at 8q23.3 (OR 1.71, 95 % CI 1.05–2.76, $p = 0.03$, dominant model) and rs3802842 at 11q23.1 (OR 1.51, 95 % CI 1.03–2.22, $p = 0.04$, dominant model). We identified two risk variants that are associated with a more severe phenotype in *APC* mutation carriers. These risk variants may partly explain the phenotypic variability in families with the same *APC* gene defect. Further studies with a larger sample size are recommended to evaluate and confirm the phenotypic effect of these SNPs in FAP.

Keywords: Familial adenomatous polyposis, Cancer genetics, Colonic adenomas, Genetic polymorphisms

INTRODUCTION

Familial adenomatous polyposis (FAP) is a hereditary colorectal cancer (CRC) susceptibility syndrome, caused by germline mutations in the adenomatous polyposis coli (*APC*) gene, which is located on chromosome 5. Carriers of mutations in the *APC* gene develop multiple colorectal adenomas and consequently have a high risk of developing CRC. The risk of CRC in these individuals is related to the number of colorectal adenomas.[1] The severity of polyposis, reflected by the number of colorectal adenomas and the age of onset, is correlated with the site of the *APC* mutation.[2] Most patients with mutations in the codon 1250–1464 region develop thousands of colorectal adenomas in the first or second decades of life. Patients with a mutation at either end or in a specific splice site region of the *APC* gene (codons <157, 312–412, >1595) usually have an attenuated polyposis phenotype, with less than a hundred polyps and an age of onset in the third or fourth decades. The majority of FAP patients have mutations in the remainder of the gene and develop hundreds to thousands of polyps from the second decade of life onwards. However, there is also phenotypic variability within FAP families with the same underlying gene defect, suggesting that beside the *APC* genotype, other factors also play a role in determining the severity of polyposis and the risk of CRC.

Both environmental and genetic factors are known to influence CRC risk.[3] To date, several single nucleotide polymorphisms (SNPs) that show an association with sporadic CRC have been identified by genome-wide association studies (GWAS).[4–10] Furthermore, gene-environmental interactions may play a role in the effect of SNPs on CRC predisposition.[11]

Two of these CRC-associated SNPs (rs16892766 and rs3802842) have been shown to be significantly associated with the risk of CRC and/or age of CRC development in patients with Lynch syndrome.[12–14]

We hypothesized that SNPs associated with sporadic CRC may play a role in polyp formation in patients with a germline *APC* mutation. In the present study, we assessed whether known CRC-associated SNPs influence the disease phenotype in patients with a germline *APC* mutation.

METHODS

Patients

A total of 419 patients from 182 families with a proven germline *APC* mutation were selected from the polyposis database of the Netherlands Foundation for the Detection of Hereditary Tumors. All patients gave informed consent for registration in the database and for use of their medical data for research purposes. All patients had also given written consent for use of their DNA in further institutional ethics-approved research into their condition before the study. The following data were collected: gender, mode of diagnosis (symptomatic or by screening), age at diagnosis of polyposis and CRC, cumulative number of colorectal adenomas, age at colorectal surgery, date and status of last follow-up. Based on the *APC* mutation site, patients were categorized into attenuated, intermediate or severe genotype groups, as described in the introduction.[2]

Genotyping of SNPs

DNA was extracted from peripheral lymphocytes using an automated procedure (Gentra Systems, Minneapolis, USA) and quantified using Picogreen (Invitrogen, California, USA). Genotyping of the SNPs was performed with the KASPar genotyping system, and outsourced to Kbioscience (http://www.kbioscience.co.uk).

Statistical analysis

The Hardy–Weinberg equilibrium of the SNPs was first tested using PLINK, version 1.07.[15] Further analyses were performed using PASW Statistics 20. The patients were categorized according to the number of colorectal adenomas. We defined two groups: the first group with less than 100 adenomas, and the second group with 100 or more adenomas. The allele frequency of the SNPs was compared between the two groups. To assess association between phenotype and SNP, genotypic odds ratios (OR) and 95 % confidence intervals (CI) were computed using the Generalized Estimating Equation, with exchangeable as working covariance structure for observations within families. A general model

for the risk alleles was used for assessing statistical significance, where a dominant model was used in case of rare alleles. As a second step, we also fitted dominant and recessive models to provide further information. For testing, Wald tests were applied. *APC* mutation site, categorized as genotype group, was included in the model as a covariate. For all statistical analysis, a *p* value of <0.05 was considered to show a trend of association. When Bonferroni multiple testing correction was applied for 15 SNPs at thirteen susceptibility loci, $p < 0.004$ should be considered as cut off point for significance.

RESULTS

A total of 419 *APC* mutation-positive patients were included, of which 188 (44.9 %) had more than 100 colorectal adenomas. The clinical and demographic characteristics of the study subjects are shown in Table 1.

Regarding differences between groups, more patients with >100 colorectal adenomas (38 %) were symptomatic on diagnosis compared to the other group (15 %). In addition, the frequency of CRC in the >100 adenoma group was significantly higher than the other group. About 75 % of patients from both phenotype groups had an intermediate phenotype but the proportion of patients with mutations belonging to the attenuated genotype group was twice as high in <100 adenoma as the >100 adenoma group (Table 1).

Of the 16 SNPs tested, fifteen SNPs were in Hardy–Weinberg equilibrium (Table 2). One SNP, rs4939827, showed borderline significant deviance and was excluded from further analyses.

The association of all 15 SNPs with disease phenotype in *APC* mutation carriers was modelled by Generalized Estimating Equilibrium with exchangeable variance structure. Allelic distribution, genotypic ORs and the corresponding 95 % CIs for each SNP are shown in Table 3 (general inheritance model) and Fig. 1 (dominant and recessive inheritance models). Due to the low number of patients with the CC genotype for rs16892766, the genotypic OR for the CC could not be estimated and therefore the dominant model was applied.

For rs16892766, carriage of the C allele showed a trend of association with a more severe phenotype (OR 1.71, 95 % CI 1.05–2.76, $p = 0.03$, dominant model).

At 11q23.1 (rs3802842), a borderline association was observed in the codominant inheritance model (Wald 2df *p* value =0.02), and when tested for the recessive and dominant models of inheritance, carriers of the risk allele of this SNP were also more frequent in the ≥100 polyp group (OR 1.51, 95 % CI 1.03–2.22, *p* = 0.04, dominant model). The other SNPs showed no associations.

TABLE 1: Clinical & demographic characteristics of 419 *APC* mutation carriers

	<100 adenomas (N=231)	***≥ 100 adenomas) (N=188)***
Gender		
Male (%)	111 (48%)	99 (53%)
Polyposis		
Mean age at diagnosis, years	26.5 (4-69)	27.6 (6-60)
Mode of diagnosis		
Symptomatic (%)	34 (15%)	72 (38%)
Screening (%)	197 (85%)	116 (62%)
CRC (%)	19 (8%)	30 (16%)
Mean age at CRC, years (range)	43.4 (26-59)	40.4 (21-60)
Mutation group		
Attenuated (%)	50 (22%)	20 (11%)
Intermediate (%)	172 (74%)	141 (75%)
Severe (%)	9 (4%)	27 (14%)
Last follow-up		
Age, years	34.7 (8-75)	40.4 (10-81)
Status at last follow-up		
Alive (%)	221 (96%)	165 (88%)
Dead due to CRC (%)	9 (4%)	14 (7%)
Dead due to other cause (%)	1(0.4%)	9 (5%)

TABLE 2: Test for Hardy-Weinberg Equilibrium

SNP	*Chromo-some region*	*Alleles Major/ Minor*	*Risk Allele*	*HWE P value*	*MAF[a] (allele)*	*Gene*	*Ref.*
rs6691170	1q41	G/T	T	0.2182	0.321 (T)	*DUSP10*	[9]
rs6687758	1q41	A/G	G	0.1461	0.160 (G)	*DUSP10*	[9]
rs10936599	3q26.2	C/T	C	0.8902	0.229 (T)	*MYNN*	[9]
rs16892766	8q23.3	A/C	C	0.5592	0.091 (C)	*EIF3H*	[4]
rs6983267	8q24.21	G/T	G	0.2798	0.461 (T)	*MYC*	[5]
rs10795668	10p14	G/A	G	0.1723	0.311 (A)	unknown	
rs3802842	11q23.1	A/C	C	0.6216	0.265 (C)	*POU2AF1*	[6]
rs7136702	12q13.13	C/T	T	0.8298	0.346 (T)	*LARP4*	[9]
rs11169552	12q13.13	C/T	C	0.6966	0.247 (T)	*DIP2B*	[9]
rs4444235	14q22.2	T/C	C	0.2362	0.432 (C)	*BMP4*	[7]
rs4779584	15q13.3	C/T	T	1	0.159 (T)	*GREM1*	[8]
rs9929218	16q22.1	G/A	G	0.4207	0.304 (A)	*CDH1*	[7]
rs4939827	18q21.1	C/T	T	0.04911	0.435 (T)	*SMAD7*	[10]
rs10411210	19q13.11	C/T	C	0.07355	0.127 (T)	*RHPN2*	[7]
rs961253	20p12.3	C/A	A	0.1397	0.311 (A)	*BMP2*	[7]
rs4925386	20q13.33	C/T	C	0.4955	0.311 (T)	*LAMA5*	[9]

[a] Minor allele frequency (MAF) in patients included in this study; Abbreviation: Ref: Reference

TABLE 3: Results for 15 CRC susceptibility SNPs in patients with ≥100 polyps and <100 polyps, under a codominant inheritance model

SNP	*Chrom-osome position*	*Geno-type*	*Total (%)*	*≥100 polyps (%)*	*Odds Ratio*	*95% CI*	*p-value Wald 1 df*	*p-value Wald 2 df*
rs6691170	1q41		410 (100)	182				0.96
		GG	195 (47.6)	86 (47.3)	1			
		TG	167 (40.7)	74 (40.7)	1.03	0.68-1.56	0.89	
		TT	48 (11.7)	22 (12.0)	1.01	0.57-2.13	0.78	
rs6687758	1q41		419 (100)	188				0.35
		AA	300 (71.6)	133 (70.7)	1			
		GA	104 (24.8)	46 (24.5)	2.42	0.71-1.87	0.56	
		GG	15 (3.6)	9 (4.8)	2.10	0.72-8.17	0.16	
rs10936599	3q26.2		410 (100)	180				0.39
		TT	21 (5.1)	10 (5.5)	1			
		TC	146 (35.6)	68 (37.8)	0.99	0.46-2.13	0.98	
		CC	243 (59.3)	102 (56.7)	0.76	0.35-1.65	0.49	
rs16892766	8q23.3		417 (100)	187				
		AA	343 (82.3)	146 (78.1)	1			
		[a]CA & CC	74 (17.7)	41 (21.9)	1.71	1.05-2.76	**0.03**	
		[CC]	[2]	[2]				
rs6983267	8q24.21		408 (100)	179				0.32
		TT	92 (22.5)	45 (25.1)	1			
		TG	192 (47.1)	84 (46.9)	0.77	0.43-1.40	0.40	
		GG	124 (30.4)	50 (27.9)	0.64	0.36-1.14	0.13	
rs10795668	10p14		417 (100)	187				0.98
		AA	33 (7.9)	14 (7.5)	1			
		GA	193 (46.3)	85 (45.4)	0.99	0.49-1.98	0.97	
		GG	191 (45.8)	88 (47.1)	0.95	0.46-1.94	0.88	
rs3802842	11q23.1		415 (100)	185				**0.02**
		AA	226 (54.5)	91 (49.2)	1			
		CA	158 (38.1)	84 (45.4)	1.70	1.13-2.55	**0.01**	
		CC	31 (7.5)	10 (5.4)	0.76	0.34-1.68	0.49	
rs7136702	12q13.13		413 (100)	185				0.65
		CC	175 (42.4)	82 (44.3)	1			
		TC	190 (46.0)	84 (45.4)	0.94	0.60-1.46	0.78	
		TT	48 (11.6)	19 (10.3)	0.75	0.42-1.37	0.35	

			415 (100)	185				0.97
rs11169552	12q13.13	CC	237 (57.1)	106 (57.3)	1			
		TC	151 (36.4)	68 (36.8)	1.01	0.61-1.68	0.97	
		TT	27 (6.5)	11 (5.9)	0.93	0.45-1.93	0.84	
			415 (100)	184				0.97
rs4444235	14q22.2	TT	128 (30.8)	57 (31.0)	1			
		CT	215 (51.8)	94 (51.1)	1.01	0.65-1.58	0.96	
		CC	72 (17.3)	33 (17.9)	0.95	0.53-1.69	0.85	
			411 (100)	183				0.27
rs4779584	15q13.3	CC	290 (70.6)	123 (67.2)	1			
		CT	111 (27.0)	57 (31.1)	1.77	0.60-5.22	0.30	
		TT	10 (2.4)	3 (1.6)	1.28	0.42-3.86	0.67	
			415 (100)	186				0.66
rs9929218	16q22.1	AA	34 (8.2)	12 (6.5)	1			
		GA	184 (44.3)	86 (46.2)	1.36	0.68-2.73	0.39	
		GG	197 (47.5)	88 (47.3)	1.22	0.59-2.52	0.60	
			418 (100)	188				0.83
rs10411210	19q13.11	TT	11 (2.6)	5 (2.7)	1			
		CT	84 (20.1)	33 (17.6)	0.90	0.25-3.22	0.88	
		CC	323 (77.3)	150 (79.8)	1.05	0.32-3.46	0.93	
			412 (100)	184				0.29
rs961253	20p12.3	CC	202 (49.0)	94 (51.0)	1			
		CA	164 (39.8)	75 (40.8)	1.00	0.62-1.63	0.99	
		AA	46 (11.2)	15 (8.2)	0.64	0.35-1.16	0.14	
			413 (100)	182				0.10
rs4925386	20q13.33	TT	43 (10.4)	18 (9.9)	1			
		TC	171 (41.4)	83 (45.6)	1.15	0.61-2.17	0.66	
		CC	199 (48.2)	81 (44.5)	0.77	0.39-1.51	0.44	

[a] Due to the low frequency, the CC genotype of rs16892766 could not be assessed; the CC and CA genotypes were combined for this SNP

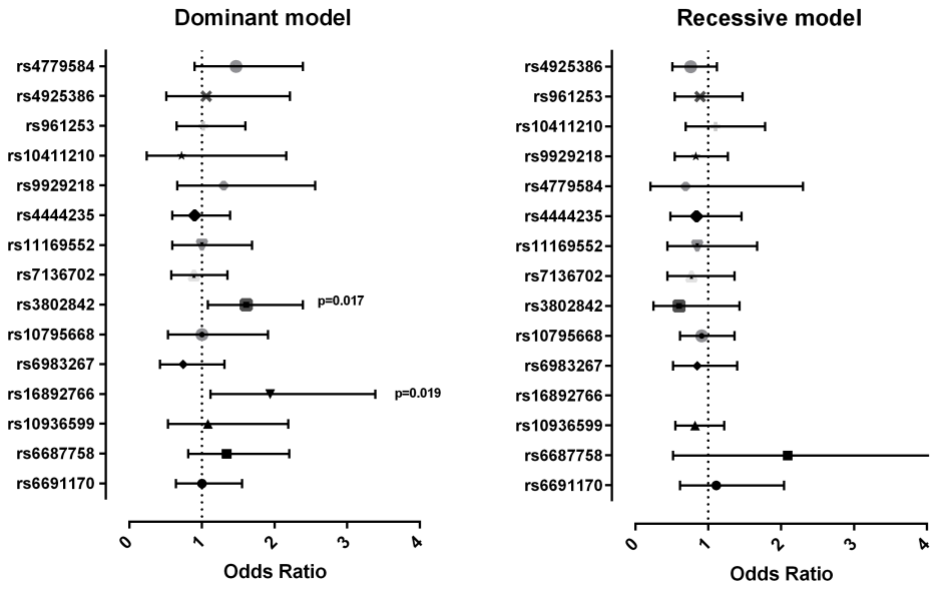

FIGURE 1: Forest plot: Results for 15 susceptibility SNPs in patients with ≥100 polyps and <100 polyps, under recessive and dominant inheritance models

When the joint association of the two SNPs (rs16892766 and rs3802842) was tested, both remained borderline significant using dominant mode of inheritance (p = 0.04 and p = 0.03, respectively), however the interaction of the two SNPs was not significant (p = 0.80).

When the total number of sporadic CRC risk alleles in individuals of both groups was compared, the mean number of risk alleles was similar (mean of 13.11 risk alleles for the <100 and 12.90 for the ≥100 group).

DISCUSSION

In this study, we examined the role of CRC-associated SNPs in disease phenotype in *APC* mutation carriers. Although a correlation between the mutation site in the *APC* gene and the phenotype of FAP is well-established,[2] the phenotypic variability observed in patients with the same underlying gene defect suggests that other factors must play a role in modifying disease expression in *APC* mutation carriers. The role of modifier genes in disease severity in FAP

patients has been investigated and several modifiers, such as N-acetyl transferases, have been suggested.[16–19]

In recent years, several SNPs have been identified that influence CRC risk in the general population. In this study, we investigated whether these SNPs influence the phenotype of patients carrying a pathogenic *APC* mutation. Two variants were found to be associated with the disease phenotype: under a dominant inheritance model, the C alleles of both rs16892766 and rs3802842 showed a trend of association with a phenotype of more than 100 adenomas.

A previous study demonstrated that individuals carrying the risk (C) allele of rs16892766 (8q23.3) present with a more advanced stage of CRC at diagnosis.[20] Tomlinson et al. found that the risk allele of rs16892766 was associated with CRC in younger individuals.[4] In other studies, the risk allele of rs16892766 correlated with an increased CRC risk and/or age of CRC diagnosis in Lynch syndrome.[12–14] In our study, the C allele of this SNP was associated with a more severe FAP phenotype (≥100 polyps) in *APC* mutation carriers. The higher polyp number associated with the C allele of rs16892766 could be explained by the location of this SNP in the *EIF3H* gene, which increases cell proliferation, growth, and survival when overexpressed. However, Carvajal-Carmona *et al*.[21] suggested that *UTP23*, rather than *EIF3H*, is the most likely target of the genetic variation associated with CRC in the 8q23.3 region, but also proposed that both of these genes may play a role in CRC development, given that they have related roles in mRNA translation. *UTP23* is thought to be involved in ribosome biogenesis.[22]

The risk allele of rs3802842 (11q23.1) has been associated with early-onset CRC (<50 years old) and a family history of CRC.[20, 23] Moreover, this SNP is also known to be associated with increased CRC risk in patients with Lynch syndrome.[12–14] A recent study described the association of rs3802842 with disease in patients with unexplained polyposis.[20, 24] In the present study, rs3802842 showed a borderline association with the more severe phenotype of ≥100 polyps in the codominant model of inheritance with two degrees of freedom. When this SNP was tested under recessive and dominant inheritance models, a trend of association was observed between risk allele carriage and the ≥100 polyp phenotype (dominant inheritance model). Functionally, rs3802842 is located within a gene-rich region

of chromosome 11q23 that includes four open reading frames (ORFs) within 100 kb: *COLCA1*, *COLCA2*, *POU2AF1* and *C11orf53* (6). The exact function of this SNP is still unknown; one study assessed whether rs3802842 might have cis-regulatory effects on these neighbouring genes, but found no evidence for a relationship. These authors suggested that the underlying sequence change defined by this SNP might exert regulatory effects on genes mapping outside 11q23.1.[25] Another study suggested that rs3802842 is not itself a functional SNP but is in linkage disequilibrium with a functional SNP.[26]

SNPs associated with CRC susceptibility could increase CRC risk by promoting initiation of adenoma formation or promoting growth and/or progression from the adenoma to carcinoma stage, or be involved in both. Theoretically, initiation-promoting SNPs are expected to be more frequent in patients with multiple adenomas and in CRC-free patients with adenoma. A recent study found eight known CRC-associated SNPs, including rs3802842, to be overrepresented in CRC-free patients with adenoma.[27] In relation to the effect of SNPs on the above-mentioned stages, only the association of a CRC-associated SNP at 8q24.21 (rs6983267) with adenoma multiplicity and the association of rs3802842 and rs4779584 with unexplained polyposis have been described to date.[6, 24] Based on these literature reports and the outcome of our study, we hypothesize that rs3802842 is involved in the initiation stage of adenoma development.

An association between the total number of CRC-associated risk alleles and familial CRC has been suggested in two previous studies.[28, 29] Therefore, we investigated whether there was a difference in total number of risk alleles between the two groups. We found the mean number of risk alleles to be similar in the two groups.

Recently, one study examined the severity of polyposis in 64 patients and found no evidence of association in any of their tested SNPs,[30] however as stated by Talseth-Palmer *et al*.[31] large cohorts are required to examine the role of modifiers in severity of disease phenotype in FAP patients.

In conclusion, we identified two CRC-associated SNPs, rs16892766 (8q23.3) and rs3802842 (11q23.1), which show an association with adenoma number in *APC* mutation carriers. In order to evaluate and confirm the effect of these

SNPs on the phenotype of FAP, further studies with larger sample sizes are now recommended.

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CHAPTER 3

Extracolonic Cancer Risk in Dutch Patients with APC (adenomatous polyposis coli)-Associated Polyposis

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ABSTRACT

Background: Screening of patients with familial adenomatous polyposis (FAP) have led to a substantial reduction in mortality due to colorectal cancer (CRC). Recent guidelines suggest that surveillance of non-intestinal malignancies should also be considered in those patients. However, the value of these surveillance programmes is unknown. The aims of this study were (1) to assess the occurrence of extracolonic malignancies in a large series of adenomatous polyposis coli (APC) mutation carriers and (2) to evaluate the causes of death.

Methods: All APC mutation carriers were selected from the Dutch polyposis registry. Data on causes of death were collected. Pathology reports were retrieved from the Dutch Pathology Registry.

Results: A total of 85 extracolonic malignancies were diagnosed in 74 of 582 APC mutation carriers. Duodenal and skin cancers were the most prevalent cancers. Thyroid cancer was observed in only 1.5% of the cases. The main cause of death was cancer (59% of all deaths), with 42% due to CRC and 21% due to duodenal cancer. One patient died from thyroid cancer. The second and third most common causes of death were cardiovascular disease (13% of all deaths) and desmoid tumours (11% of all deaths), respectively.

Conclusion: Extending surveillance programmes to other cancers will not contribute significantly to the survival of patients with FAP.

Keywords: APC mutation; Extracolonic cancer; FAP; Familial adenomatous polyposis.

INTRODUCTION

Familial adenomatous polyposis (FAP) is an autosomal dominantly inherited disease caused by germline mutations in the adenomatous polyposis coli (APC) gene.[1] FAP is characterised by the development of hundreds to thousands of colorectal adenomas, and unless prophylactic colectomy is performed the risk of colorectal cancer (CRC) is almost 100%. While nearly all mutation carriers also develop duodenal adenomas, the risk of duodenal cancer is significantly lower, estimated at around 5% to 15%.[2-3] Less frequently, other extracolonic cancers may be observed such as cancers of the thyroid, brain, stomach, pancreas, liver and desmoid tumours. Desmoid tumours are histologically benign fibroblastic tumours that are locally aggressive but lack metastatic potential.

During the 1980s and 1990s, polyposis registries were established with the aim of promoting participation in colorectal and upper gastrointestinal (GI) surveillance programmes to prevent intestinal cancer. Establishment of these registries has resulted in earlier diagnosis and significant decrease in incidence of CRC, and an increase in the life expectancy of patients with FAP.[4–6] As a consequence, studies began to report a shift in the causes of death in those patients and today, beside CRC, the main reported causes of death are duodenal cancer and desmoid tumours.[7-8]

An important and still unanswered question is whether life expectancy of patients with FAP can be further improved by extending existing surveillance programmes to other organs such as the thyroid, stomach, liver or pancreas. Decisions on surveillance of a particular organ should be based on the criteria proposed by Wilson and Jungner.[9] Most important among these criteria are the following requirements: (1) the disease should be a common manifestation in the target group, (2) available screening tools should be highly sensitive and specific (and not too burdensome), and (3) early treatment should improve the prognosis and increase life expectancy.

Previous studies have reported risks that vary widely for cancers of the thyroid, liver and pancreas in FAP. As a consequence, there is still no consensus regarding the need for additional surveillance recommendations for these cancers.

The aims of this study were (1) to assess the occurrence of extracolonic malignancies and benign tumours in a large series of patients with FAP with a proven APC mutation known from the Dutch polyposis registry and (2) to evaluate whether these extracolonic malignancies are an important cause of death.

PATIENTS AND METHODS

Dutch Polyposis Registry

The Dutch Polyposis Registry was established by the Netherlands Foundation for Detection of Hereditary Tumours (NFDHT) in 1985. The main objective of the registry is to promote early detection of upper and lower GI cancers through coordination of lifelong surveillance of at-risk patients.[10] Patients with FAP are referred to this national registry by gastroenterologists, surgeons or clinical geneticists. At the time of registration, written informed consent for collection of personal and medical data is provided by all patients. At regular intervals (usually annually), the registry sends out reminders to gastroenterologists to prompt patient screening, and the results of these endoscopies and results of histological examinations are then sent to the registry. To date, >2000 patients with a clinical diagnosis of FAP have been registered, of which >500 are proven APC germline mutation carriers. This study was approved by the research committee of the registry.

Cross-referencing the Dutch Polyposis Registry to the National Pathology Registry

For this study, we selected all patients with FAP with a proven germline APC mutation in the Dutch polyposis registry. All medical information, age at last follow-up and mortality including cause of death and age at death were collected. The medical files usually contain histology reports of surgical specimens or biopsies. However, to improve the quality of the medical data, we cross-referenced polyposis registry data to the 'nationwide network and registry of histopathology and cytopathology in the Netherlands (PALGA)', which was established in 1971.[11] The PALGA database does not contain identifying patient

data, but only pseudonyms based on this data. Pseudonymisation of the Dutch polyposis registry was carried out by a trusted third party to maintain data confidentiality.

Statistical analysis

Descriptive statistical analysis was used. Observations were from 1971 or time of registration to death, loss to follow-up or end of the study (1 May 2014). Data were analysed and calculations were carried out using SPSS V.22.0.

RESULTS

Study population

A total of 567 proven APC mutation carriers registered at the Dutch Polyposis Registry were enrolled in the study. All included patients were cross-referenced to the PALGA registry. Of those, two patients were excluded due to missing clinical data. An additional search in the polyposis registry identified a further 17 patients with FAP with a confirmed APC mutation who were also included in the study. Using the records of the Dutch Polyposis Registry, the occurrence of extracolonic malignancies was studied in these 17 patients. The total group of patients was 582 (49.7% male). The mean age at last follow-up was 39.9 years.

Benign extracolonic lesions

Of the 565 patients initially enrolled in the study, 191 (33.8%) had at least one pathology report with evidence of duodenal adenomatous polyposis. Fundic gland polyposis was reported in 102 (18.1%) patients, and 24 (4.2%) patients had at least one gastric adenoma, all located in the antrum.

Desmoid tumours confirmed by a histology report were described in 27 (4.8%) of the carriers. A total of 38 patients (6.7%) had a benign skin tumour of several types. Lipomas and osteomas were documented in 11 (1.9%) and 7 (1.2%) patients, respectively. Other reported tumours included a benign thyroid tumour (one patient), adrenal tumours (two patients) and ovarian cystadenomas (three patients).

Malignant extracolonic lesions

A total of 85 extracolonic malignancies were documented by pathology report in 582 APC mutation carriers, and 74 (12.7%) of the carriers had at least one malignant extracolonic lesion. The frequency of extracolonic malignancies and mean age at diagnosis are shown in table 1.

TABLE 1. Extracolonic cancers in 582 FAP patients with APC-mutation

Site of Cancer	*N (%)*	*Gender (M/F)*	*Mean age at diagnosis (range)*
Duodenum	18 (3.1)	8/10	52.1 (32-79)
Skin	13 (2.2)	8/5	52.1 (26-67)
Thyroid	9 (1.5)	1/8	38.6 (19-53)
Lung	7 (1.2)	4/3	50.1 (35-61)
Breast	6 (2.0) *	0/6	43.5 (33-68)
Bladder	4 (0.7)	3/1	51.5 (31-64)
Hepatoblastoma	4 (0.7)	3/1	1.8 (1-3)
Unknown primary	4 (0.7)	3/1	42.5 (23-55)
Liver	3 (0.5)	3/0	31 (17-68)
Endometrium	2 (0.7) *	0/2	44.5 (41-48)
Brain tumour	2 (0.3)	1/1	31.0 (13-49)
Meningioma	2 (0.3)	1/1	40.0 (46-34)
Pancreas	2 (0.3)	1/1	54.5 (40-69)
Others †	9 (1.5)	5/4	46.6 (26-77)

*% calculated in female patients.
†Ovary, cervix, vagina, lymphoma, larynx, vocal cords, parotid, prostate, stomach.
APC, adenomatous polyposis coli; FAP, familial adenomatous polyposis; m/f, male/ female.

Duodenal cancer and skin cancers (basal cell carcinoma, squamous cell carcinoma and melanoma) were the most common extracolonic cancers (in 3.1% and 2.2% of patients, respectively), followed by thyroid cancer (1.5%). Six of 294 (2.0%) female patients were diagnosed with breast cancer, at a mean age of 43.5

years. In four of these patients, the breast cancer was diagnosed before 40 years of age.

Thyroid cancer was documented in nine patients (1.5%). Seven female patients (2.4% of all female patients) were diagnosed with papillary thyroid cancer, with a mean age of 33.5 years at diagnosis. Duodenal cancer was observed in 18 patients including 14 of ampullary origin (3.1%), hepatoblastoma in 4 (0.7%), bladder cancer in 4 (0.7%) and pancreatic malignancy, malignant brain tumour and meningioma were each observed in 2 patients.

Cause of death

In addition to CRC (25% of all deaths), extracolonic cancers (33.9%) and cardiovascular disease (12.5%) were the two most common causes of death in Dutch APC mutation carriers. Duodenal cancers and desmoid tumours were responsible for 12.5% and 10.7% of deaths, respectively. One patient died from thyroid cancer at the age of 78 years. Two patients died due to complications of Whipple surgery and one patient committed suicide at the age of 36 years. Table 2 describes the causes and age at death in APC mutation carrier patients in the Dutch polyposis registry.

TABLE 2. Cause of death in APC mutation polyposis patients

	N (%)	*Gender (M/F)*	*Mean age at Death (range)*
CRC	14 (25)	5/9	46.8 (23.4-65.7)
Other Cancers*	19 (33.9)	10/9	51.6 (33.0-78.4)
Cardiovascular Disease	7 (12.3)	6/1	68.1 (82.3-45.1)
Desmoid Tumour	6 (10.7)	4/2	40.5 (33.0-49.3)
Others	4 (7.1)	2/2	56.2 (30.4-80.2)
Unknown	6 (10.7)	2/4	49.9 (36.3-65.0)
Total	56 (100)	29/27	51.2 (23.4-82.6)

*Known FAP association: duodenal cancer (7), pancreatic cancer (2), brain tumour (1), thyroid cancer (1); not known FAP association: lung cancer (3), unknown primary (2), gastric cancer (1), non-Hodgkin's lymphoma (1), hepatocellular carcinoma (1).

APC, adenomatous polyposis coli; CRC, colorectal cancer; FAP, familial adenomatous polyposis; m/f, male/female.

DISCUSSION

The most common extracolonic cancers found in this study were duodenal cancer and skin tumours. The frequency of other FAP-associated cancers such as cancer of the thyroid, liver (hepatoblastoma), brain and stomach was low. Among the benign lesions, the most frequent were fundic gland polyps, duodenal and gastric adenomas and desmoid tumours. The most prevalent cause of death was cancer (59%), with 42% of the cancer deaths due to CRC and 21% of the cancer deaths due to duodenal cancer. The second and third most common causes of death were cardiovascular disease (12.5% of all deaths) and desmoid tumours (10.7% of all deaths), respectively.

A number of studies have reported frequencies for extracolonic cancers in FAP, but results vary widely. For instance, the prevalence rates of thyroid cancer in the literature vary from 0.4% to 11.8%.[12–14] Reported frequencies appear to depend on the type of study, that is, prospective versus retrospective studies or large registry studies versus small series. The frequencies found in our study were similar to those reported in previous registry-based studies (1%–2%). Likewise, the frequencies of brain tumours, pancreatic cancer and hepatoblastoma we observed were also equal to rates reported in the literature at around <1%–2%.[15-16]

Prevalence of duodenal cancer was relatively low at 3%, which may be due to upper GI surveillance and the implementation of prophylactic duodenectomy in patients with advanced duodenal polyposis.[17] In this study, we identified only one patient with gastric cancer, in agreement with earlier studies that showed that the incidence of this cancer is not increased in FAP families in Western countries. By contrast, studies from Japan and South Korea report gastric cancer rates of 3%–4%.[18-19]

Although the incidence of other cancers (eg, cancer of lung and breast) was not increased, interestingly, the age of breast cancer diagnosis observed in our patients with polyposis was relatively low.

One of the strengths of this study was that we were able to assemble a very large cohort of proven APC mutation carriers with a follow-up of more than 25 years. In addition, cross-referencing the polyposis registry with the PALGA database

allowed us to expand the number of patients with pathologically confirmed tumours.

The limitation of the study was that some extracolonic tumours such as brain tumours and desmoid tumours were not always confirmed by pathology and therefore may have been underestimated. The same is probably true for some benign lesions, such as fundic gland polyps, because the endoscopic appearance of fundic gland polyposis in a patient with FAP is very typical and biopsies for pathological confirmation are often not obtained. Furthermore, the PALGA database was established in 1971 and gradually extended its coverage to 100% by 1991. As a consequence, some tumours might have been missed.

What are the implications of this study for clinical practice? The consensus in the current guidelines is that patients should be screened for duodenal adenomas/cancer, in addition to surveillance of the colon. However, consensus has not yet been reached with respect to other non-intestinal cancers, including cancers of the thyroid, pancreas and liver.[15, 20-21]

If we apply the above-mentioned criteria developed by Wilson and Jungner to decision-making on thyroid cancer surveillance, we can conclude that the risk of thyroid cancer found in this study and other registry studies is relatively low, with women showing the highest risk. Although highly sensitive screening tools for thyroid cancer like ultrasonography (US) and fine needle aspiration (FNA) biopsy are available, a major disadvantage is that regular examination will frequently reveal benign lesions that result in FNA biopsy. These findings and subsequent biopsies represent a burden for patients and may also cause substantial anxiety. The most important finding of our study was that only one patient died from this cancer, at the age of 78 years. On the basis of these considerations, we do not recommend thyroid cancer surveillance in patients with FAP outside of research programmes.

Regarding the need for liver tumour (hepatoblastoma) surveillance, the prevalence of this type of tumour in our and other studies was very low, at around 1%. In addition, none of the four patients in our study died as a result from this tumour. Because hepatoblastoma is a disease that occurs in early childhood, a consequence of the decision to screen mutation carriers for these

tumours would be that APC mutation analysis would have to be performed at birth rather than from the age of between 10 and 12 years, as is current practice. This change in policy would lead to stigmatisation of children found to be mutation carriers. In view of these considerations, we do not recommend surveillance of mutation carriers for liver tumours outside of research programmes.

Finally, although cancer of the pancreas appears to be associated with FAP, the prevalence is very low and we do not recommend screening.

In conclusion, the life expectancy of patients with FAP has increased substantially following implementation by registries of screening programmes for colorectal and duodenal cancer. On the basis of our data, we conclude that extending these surveillance programmes to extraintestinal-associated cancers will not further improve life expectancy in APC mutation carriers.

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PATIENT CONSENT

Patients registered at the Netherlands Foundation for the Detection of Hereditary Tumors (StOET) sign a consent form approving the use of their medical data for research purposes upon registration.

ETHICS APPROVAL

Approved by boards of the Netherlands Foundation for the Detection of Hereditary Tumors (StOET) and the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA).

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CHAPTER 4

Increased Prevalence of Barrett's Esophagus in Patients with MUTYH-Associated Polyposis (MAP)

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ABSTRACT

Barrett's oesophagus (BE) has been associated with an increased risk of both colorectal adenomas and colorectal cancer. A recent investigation reported a high frequency of BE in patients with adenomatous polyposis coli (*APC*)-associated polyposis (FAP). The aim of the present study is to evaluate the prevalence of BE in a large cohort of patients with *MUTYH*-associated polyposis (MAP) and *APC*-associated adenomatous polyposis. Patients with a genetically confirmed diagnosis of familial adenomatous polyposis (FAP) or MAP were selected and upper gastrointestinal (GI) endoscopy reports, pathology reports of upper GI biopsies were reviewed to determine the prevalence of BE in these patients. Histologically confirmed BE was found in 7 (9.7%) of 72 patients with MAP. The mean age of diagnosis was 60.2 years (range 54.1–72.4 years). Two patients initially diagnosed with low grade dysplasia showed fast progression into high grade dysplasia and esophageal cancer, respectively. Only 4 (1.4%) of 365 patients with FAP were found to have pathologically confirmed BE. The prevalence of BE in patients with MAP is much higher than reported in the general population. We recommend that upper GI surveillance of patients with MAP should not only focus on the detection of gastric and duodenal adenomas but also on the presence of BE.

Keywords: Barrett's esophagus, *MUTYH*-associated polyposis, Familial adenomatous polyposis, Esophageal adenocarcinoma

INTRODUCTION

The incidence of esophageal adenocarcinoma (EAC) in Western populations has substantially increased over the past several decades. The majority of EACs is thought to derive from a precursor lesion—Barrett esophagus (BE). BE is characterized by the presence of columnar epithelium that has replaced the normal squamous cell lining of the distal esophagus. EAC develops through multistep progression from metaplasia into low grade dysplasia, high grade dysplasia, early adenocarcinoma, and, finally, invasive cancer. This metaplastic change is driven by chronic inflammation due to gastro-esophageal reflux disease (GERD), which is aggravated by abdominal obesity and smoking.[1, 2] In addition to environmental factors associated with BE and EAC, also genetic factors are thought to play a role.[3, 4]

The prevalence of BE in asymptomatic patients varies between 0.5 and 1.8% and in patients with reflux symptoms, between 1.5 and 12.3%.[5–9] (Table 1). It has been reported that BE and EAC are associated with a higher incidence of (sporadic) colorectal adenomatous polyps.[10] Also, familial adenomatous polyposis (FAP) caused by germline mutations in the adenomatous polyposis coli (*APC*) gene, has been associated with an increased risk of developing BE.[11, 12] It is not known whether adenomatous polyposis caused by bi-allelic germline mutations in the *MUTYH* gene (*MUTYH*-associated polyposis (MAP)) is also associated with BE.[13] The *MUTYH* gene plays an important role in base excision repair. Base excision repair is a cellular mechanism that repairs damaged DNA throughout the cell cycle. It is responsible primarily for removing small, non-helix-distorting base lesions from the genome.[14] EAC has been reported as part of the extracolonic tumor spectrum of MAP.[15]

Surveillance of the upper gastrointestinal (GI)-tract is recommended for patients with MAP and FAP because of the increased risk of gastric and duodenal adenomas.[16] In the present study we assessed the prevalence of BE and EAC by reviewing the endoscopy reports in a large cohort of patients with FAP and MAP.

TABLE 1. The prevalence of Barrett's esophagus (BE) reported in the general population (GP) and patients with and without gastro-esophageal reflux disease (GERD) symptoms

Study	*Year*	*Country*	*Total number of pts*	*Prevalence of BE* in GP (%)*	*Prevalence of BE in pts with GERD** (%)*	*Prevalence of BE in pts without GERD (%)*
Ronkainen *et al*.	2005	Sweden	1000	1,6	2,3	1,2
Zagari *et al*.	2008	Italy	1033	1,3	1,5	1,0
Peng *et al*.	2009	China	2580	1,0	-	0,5
Lee *et al*.	2010	South Korea	2048	1,0	12,3	0,5
Zou *et al*.	2011	China	1030	1,8	2,1	1,8

METHODS

Initially, the database of the Department of Genetics at the University Medical Centre Utrecht was used to identify patients diagnosed with a polyposis syndrome between November 1987 and April 2015. Patients with a genetically confirmed diagnosis of FAP or MAP were eligible for this study if one or more upper GI endoscopy reports and/or pathology reports of upper GI biopsies were available.

To increase the number of FAP and MAP patients, we also used data from the Dutch Hereditary Cancer Registry. This national registry, established in 1985, collect medical and pathology reports and reports of upper and lower GI endoscopy of all registered patients with FAP and MAP.

All available original upper-GI endoscopy reports were reviewed. Data on the presence of BE, length of BE and, if available, the Prague criteria (endoscopic grading system for BE) were recorded. Also, the presence of a hiatal hernia and GERD (based on the Los Angeles, LA classification[17]) were obtained. Secondly, the pathology reports of all included patients were collected from the PALGA (Dutch acronym for “Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief”) database to confirm the histological diagnosis of BE. The PALGA database is a national automated archive where all pathology reports of all

performed biopsies in the Netherlands are registered. BE was defined as esophageal columnar epithelium in the presence of goblet cells.[18] The available section slides of the Barrett biopsies were reviewed by an expert GI pathologist (GJHO and MML).

Only patients from the Department of Clinical Genetics that have given their informed consent for their medical records to be reviewed were included.

All patients registered at the Dutch Hereditary Cancer Registry have given written informed consent for registration and use of their anonymous data for research.

Descriptive statistical analysis was used. Frequencies are presented as absolute numbers and percentages. Continuous data are presented as mean [standard deviation (SD)], and in the case of non-normally distributed data as median (range). Last follow-up was calculated as death, diagnosis of BE or end of the study.

RESULTS

Prevalence of BE in patients with MAP

A total of 94 patients with genetically proven MAP were selected. In 72 of the 94 MAP patients, the upper GI endoscopy reports and/or pathology reports of upper GI biopsies were available, including 28 females and 44 males. The mean age at last follow-up was 60.9 years (range 27.3–87.6, SD 11.4) and the mean length of follow-up (in 60 of 72 MAP patients where data was available) was 10.1 years (range 0–26.2). Patients characteristics and endoscopic findings are shown in Table 2.

TABLE 2. Frequency of endoscopic findings in the esophagus in MAP and FAP patients

		MAP patients (%)	*FAP patients (%)*
Total number of patients		72	356
Gender	Female	28 (38.9%)	179 (50.3%)
	Male	44 (61.1%)	177 (49.7%)
Age at last follow-up (range)		60.9 (27.3-87.6)	48.9 (30.3-86.0)
Endoscopic findings esophagus			
Histologically proven Barrett's mucosa		7 (9.7%)	4 (1.4%)
Esophagus adenocarcinoma		1 (1.4%)	0
Other findings			
Gastro-esophageal reflux esophagitis		18 (25%)	NA
Hiatal hernia		10 (14%)	NA

MAP=*MUTYH*-associated polyposis; FAP=*APC*-associated polyposis; NA=not available

A total of nine patients had an endoscopical diagnosis of BE, and in seven out of the nine patients, BE was confirmed by histology (Table 3). Revision of the section slides by an expert pathologist was possible in six out of seven patients, and in all six patients the diagnoses of BE was confirmed. Thus, the prevalence of pathologically confirmed BE in the total cohort was 9.7% (7/72). The seven patients with BE included five males and two females. The mean age at diagnosis of BE was 60.2 years (range 54.1–72.4 years, SD 6.5). The characteristics of the seven patients with BE are summarized in Table 3.

Information on previous endoscopies was available in six out of the seven patients with BE. In three patients, BE was diagnosed at the first upper-GI endoscopy. In the remaining three patients, the previous endoscopy, performed 1–4 years earlier, did not demonstrate evidence for BE.

At time of diagnosis, two patients had low-grade dysplasia. The first patient developed high grade dysplasia after having low grade dysplasia in previous biopsies. The treatment consisted of piecemeal endoscopic mucosal resection. The second patient, initially diagnosed with low grade dysplasia, developed adenocarcinoma after 6 months and underwent a surgical resection of a pT2N2Mx EAC. The follow-up of the patients with BE are shown in Fig. 1.

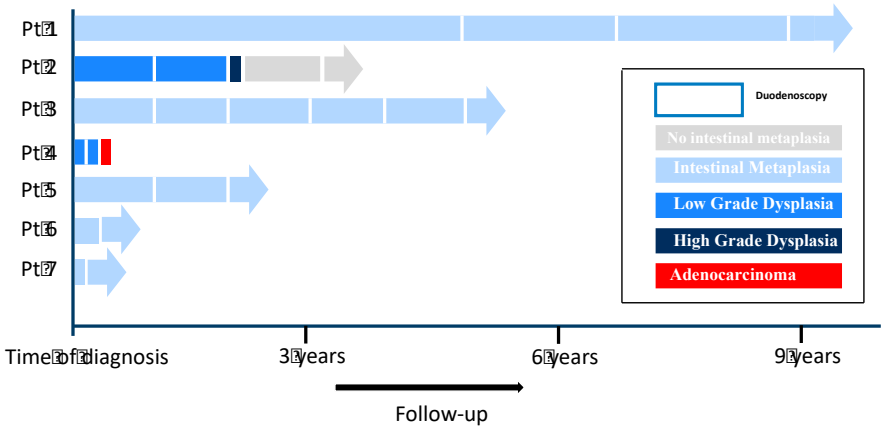

FIGURE 1. Findings at follow-up upper GI-endoscopies in the seven patients with Barrett's esophagus. Arrow blocks represent screening intervals and the colors indicate the stage of metaplasia/dysplasia.

Prevalence of BE in patients with (A)FAP

In total, 407 FAP patients were identified from the 2 datasets. Upper GI endoscopy reports and/or pathology reports of upper GI biopsies were available in 356 patients including 177 males and 179 females. The mean age at the last follow-up was 48.9 years (range 30.3–86.0, SD 11.8).

In the total cohort, five patients with BE were detected. In four of these patients including two males and two females the diagnosis was confirmed by histological examination. In the fifth patient the diagnosis could not be confirmed as no goblet cells were present in the biopsies. The prevalence of histologically proven BE in this cohort is, therefore, 1.4%. The mean age at diagnosis of the four patients with BE was 52.5 years (range 34.0–60.0, SD 12.4). The endoscopic findings are summarized in Table 2.

TABLE 3. Clinical, genetic and pathological characteristics of 7 MAP patients with Barrett's esophagus

Pt	*Sex*	*Age at dx (yrs)*	*Mutation 1*	*Mutation 2*	*Initial PA report*	*Revision*
1	F	61	c.536A>G p.(Tyr179Cys)	c.638C>T p.(Pro213Leu)	No dysplasia	No dysplasia
2	M	72	c.1147delC, p.(Glu369Argfs*39)	c.1214C>T p.(Pro405Leu)	High grade dysplasia	High grade dysplasia
3	F	54	c.1187G>A p.(Gly396Asp)	c.1214C>T p.(Pro405Leu)	Low grade dysplasia	No dysplasia
4	M	58	c.536A>G p.(Tyr179Cys)	c.536A>G p.(Tyr179Cys)	Low grade dysplasia	Not available
5	M	57	c.536A>G p.(Tyr179Cys)	c.1214C>T p.(Pro405Leu)	No dysplasia	Indefinite for dysplasia
6	M	55	c.536A>G p.(Tyr179Cys)	c.933 + 3A >C splice site intron 10	No dysplasia	Indefinite for dysplasia
7	M	65	c.536A>G p.(Tyr179Cys)	c.1187G>A p.(Gly396Asp)	No dysplasia	No dysplasia

Abbreviations: pt= patient; dx: diagnosis

DISCUSSION

The present study demonstrated a prevalence of BE (9.7%) in MAP patients which is > 5 times higher than reported in the general population. In contrast with a previous study, no increased frequency of BE was found in a large series of FAP-patients.

The prevalence of BE depends on which population is screened. In asymptomatic patients that undergo an upper GI endoscopy the prevalence varies between 0.5 and 1.8% and in patients with reflux symptoms, it is between 1.5 and 12.3% (Table 1).[5–9] The proportion of MAP patients in our series with gastro-esophageal reflux esophagitis (25%) is not higher than reported in the general population which suggests that the frequency of BE is not increased by selection of symptomatic patients.

Another interesting finding was that in this small cohort of BE patients, two of the seven patients with initial low-grade dysplasia showed fast progression to high grade dysplasia and EAC, respectively. From a biological point of view our findings seem plausible. Persistent inflammation in esophageal mucosa due to acids and bile acids is associated with DNA impairment caused by increased formation of reactive oxygen species (ROS).[19–21] One of the main defensive mechanisms to eliminate ROS induced DNA damage in cells is base-excision repair. Since *MUTYH* protein is a key player in base-excision repair, loss of the *MUTYH*-proteins could lead to accumulation of mutations and finally drive oncogenesis.

Analysis of our cohort of 356 FAP patients revealed that the prevalence of BE (1.4%) is not higher than in the general population. This is in contrast with a previous report on 36 (A)FAP patients of whom 6 (16%) had histologically proven BE.[11] We do not have an explanation for the observed differences but in view of the relatively small number of patients in the previous report, the findings might be due to chance. The fact that EAC has only been reported as part of the tumor spectrum of MAP but not in FAP supports our findings.

The strength of this study is the large number of patients with MAP and FAP and the long follow-up time.

In addition, all pathology reports were cross linked with the National Database (PALGA) and all biopsies of patients with BE were reviewed by an expert pathologist. There are also some limitations. At first, it is a retrospective analysis which might have led to selection of patients with BE. Secondly, not all risk-factors for the development of BE could be collected, such as smoking, obesity, symptoms of GERD or alcohol use.

What is the clinical implication of our study? Based on our observations, we recommend that upper GI surveillance of patients with MAP should not only focus on the identification of gastric and duodenal adenomas but also on the presence of BE. In view of the observed acceleration of high-grade dysplasia and EAC development, more intensive follow-up might be considered in patients with BE. In conclusion, this study demonstrates that the prevalence of BE with patients with MAP is much higher compared to the general population. This can

be explained by the impaired *MUTYH* protein function that plays a role in the repair of DNA damage caused by oxidative stress such as GERD.

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4

PART II

Constitutional Mismatch Repair Deficiency (CMMRD)

CHAPTER 5

Guidelines for Surveillance of Individuals with Constitutional Mismatch Repair Deficiency Proposed by the European Consortium "Care for CMMRD" (C4CMMRD)

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ABSTRACT

Lynch syndrome (LS) is an autosomal dominant disorder caused by a defect in one of the DNA mismatch repair genes: MLH1, MSH2, MSH6 and PMS2. In the last 15 years, an increasing number of patients have been described with biallelic mismatch repair gene mutations causing a syndrome referred to as 'constitutional mismatch repair-deficiency' (CMMRD). The spectrum of cancers observed in this syndrome differs from that found in LS, as about half develop brain tumours, around half develop digestive tract cancers and a third develop haematological malignancies. Brain tumours and haematological malignancies are mainly diagnosed in the first decade of life, and colorectal cancer (CRC) and small bowel cancer in the second and third decades of life. Surveillance for CRC in patients with LS is very effective. Therefore, an important question is whether surveillance for the most common CMMRD-associated cancers will also be effective. Recently, a new European consortium was established with the aim of improving care for patients with CMMRD. At a workshop of this group held in Paris in June 2013, one of the issues addressed was the development of surveillance guidelines. In 1968, criteria were proposed by WHO that should be met prior to the implementation of screening programmes. These criteria were used to assess surveillance in CMMRD. The evaluation showed that surveillance for CRC is the only part of the programme that largely complies with the WHO criteria. The values of all other suggested screening protocols are unknown. In particular, it is questionable whether surveillance for haematological malignancies improves the already favourable outcome for patients with these tumours. Based on the available knowledge and the discussions at the workshop, the European consortium proposed a surveillance protocol. Prospective collection of all results of the surveillance is needed to evaluate the effectiveness of the programme.

Key Words: CMMRD, Constitutional mismatch repair deficiency, surveillance, guidelines, tumour spectrum

INTRODUCTION

Lynch syndrome (LS) is an autosomal dominant disorder caused by a heterozygous defect in one of the DNA mismatch repair (MMR) genes, that is, *MLH1*, *MSH2* (and *EPCAM* deletion-mediated *MSH2* methylation), *MSH6* or *PMS2*. Carriers of a MMR defect have a high risk of developing colorectal cancer (CRC), endometrial cancer and various other cancers, most of which are diagnosed between the ages of 40 years and 60 years.[1] In the last 15 years, an increasing number of patients have been described with biallelic MMR gene mutations in which MMR defects are inherited from both parents. This leads to a syndrome with recessive inheritance, which is referred to as 'constitutional mismatch repair-deficiency' (CMMRD). The spectrum of cancers observed in patients with this syndrome differs from the spectrum found in LS,[2] as about half develop brain tumours (BTs), around half develop digestive tract cancers and a third develop haematological malignancies. LS-associated tumours such as endometrial and urinary tract cancers also occur. A large proportion (up to 40%) of patients with CMMRD, even more than in LS, develop metachronous second malignancies.[2] The prognosis of CMMRD is much worse than that of LS due to the type of malignancies that occur and the high risk of second primary tumours. BTs and haematological malignancies are mainly diagnosed in the first decade of life, and CRC and small bowel cancer (SBC) in the second and third decades of life. Endometrial cancers and urinary tract cancers are diagnosed in young adult patients with CMMRD. A variety of non-malignant lesions may also be observed in CMMRD, such as cafe au lait spots and other signs reminiscent of neurofibromatosis type-I, hypopigmentation, mild immunoglobulin deficiencies and congenital malformations.

Surveillance for CRC in patients with LS is very effective, as regular colonoscopy has been shown to reduce CRC-associated mortality by more than 60%.[3] Therefore, an important question is whether surveillance for the most common CMMRD-associated cancers might also be effective. In view of the diverse nature of the malignancies associated with the syndrome, it is not clear whether early detection is possible and will improve the prognosis.

Recently, Durno *et al* [4] published the outcome of a surveillance programme of two sisters with CMMRD. Fifteen tumours were detected over a follow-up period of 10 years, including a jejunal carcinoma and a small asymptomatic anaplastic astrocytoma that could be completely resected. Sjursen *et al* [5] reported on a patient who was followed with upper and lower endoscopy, CT and MRI at regular intervals over a period of 26 years. During this time six adenocarcinomas (of the left colon, the duodenum, the distal ileum and the proximal ileum, the proximal jejunum and the endometrium, respectively), as well as several polyps of the large and small bowels and the stomach were removed.

More than four decades ago, a WHO proposal defined the criteria that should be met prior to implementation of large-scale population screening.[6] These criteria can also be applied in the assessment of surveillance of individuals with a genetic predisposition to cancer. The most important criteria include: (A) cancer should be a common problem in the group targeted for surveillance; (B) the natural course of the cancers should be known; (C) screening tests with high sensitivity and specificity should be available and the tests should be acceptable to the patient; (D) an effective treatment should be available following detection of a tumour; (E) there should be evidence that screening leads to diagnosis of cancer at an early stage and to an improvement in prognosis; and finally, (F) the surveillance protocol should be cost-effective.

Recently, a new European consortium was established with the aim of improving care for patients with CMMRD. At a workshop of this collaborative group held in the Saint-Antoine Hospital, Paris, (9th of June, 2013), one of the issues addressed was the development of surveillance guidelines. A total of 20 experts in the field, including human and clinical geneticists, pathologists and paediatric oncologists from five countries, participated in the meeting. Experts in the field not present at the meeting were also involved in the discussions on surveillance. In this manuscript, the most important WHO surveillance criteria are addressed, and then applied to assess surveillance in CMMRD. Based on the outcome of the workshop and the recommendations of European experts, the consortium now proposes a surveillance programme. In view of the lack of studies other than case

reports, we did not use a system to grade the category of evidence of reported studies and/or strength of the recommendations.

WHAT IS THE TUMOUR SPECTRUM IN CMMRD?

A total of 91 families with CMMRD, including 146 patients, were identified in the world literature (Wimmer *et al*, in preparation). The most frequent underlying gene defects were *PMS2* mutations, which were reported in approximately 60% of cases. The remaining 40% of cases were equally distributed among *MSH6* and *MLH1/MSH2* biallelic mutation carriers. The various cancers observed are summarised in table 1.

TABLE 1. Overview of the most common cancers observed in 146 patients with CMMRD (Wimmer *et al*., in preparation)

Type of tumours	*Number*
Haematological malignancies	
NHL and other lymphoma	32
ALL	9
AML	5
other	2
Brain tumours	
Glioblastoma and other high-grade gliomas	58
(S)PNET	8
Medulloblastoma	7
Other	8
Lynch syndrome-associated tumours	
CRC	59
Small bowel cancers	18
Endometrial cancers	6
other cancers	5

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukemia;
CMMRD, constitutional mismatch repair-deficiency;
CRC, colorectal cancer; NHL, non-Hodgkin lymphoma;
(S) PNET, (supratentorial) primitive neuroectodermal tumours

BTs and digestive tract cancers were the most common cancers, identified in 53% and 40% of patients, respectively. Among the BTs, the most common cancer was high grade glioma, followed by primitive neuroectodermal tumours (PNETs) and medulloblastoma. Glioblastomas and other high-grade gliomas were much more frequent in CMMRD than would be expected based on the relative contribution of these BTs to total paediatric malignancies in the general population. Digestive tract cancers included CRC (40% of all CMMRD cases) and SBC (12% of all cases). Haematological cancers were observed in 31% and included mainly T cell non-Hodgkin lymphoma (NHL) and acute leukaemia. Other LS-associated cancers (endometrial cancer, urinary tract cancer) were also observed in adults. In addition, a large variety of other tumours were found (table 2). The distribution of ages at diagnoses for BT, haematological malignancies, CRC and SBC reported in the literature are shown in figures 1-4. It should be emphasised that the age distribution is biased by collection of published cases.

TABLE 2. Other tumours observed in 146 CMMRD patients (Wimmer *et al*., in preparation)

• Neuroblastoma
• Wilms tumour
• Ovarian neuroectodermal tumour
• Infantile myofibromatosis
• Rhabdomyosarcoma
• Basal cell carcinoma
• Muco-epidermoid carcinoma of the parotis
• Osteosarcoma

CMMRD, constitutional mismatch repair-deficiency

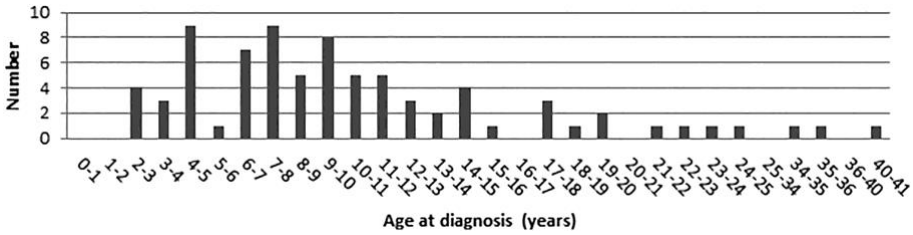

FIGURE 1. Age at diagnosis of brain tumours

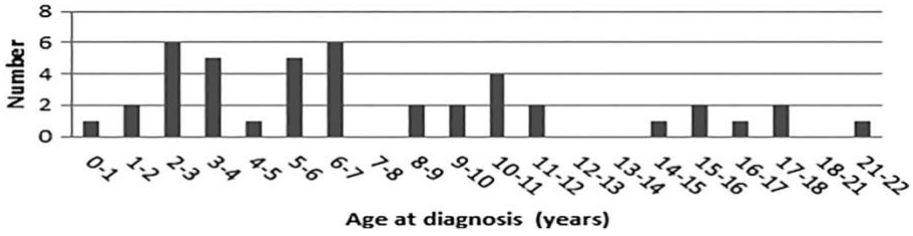

FIGURE 2. Age at diagnosis of lymphoma/leukaemia

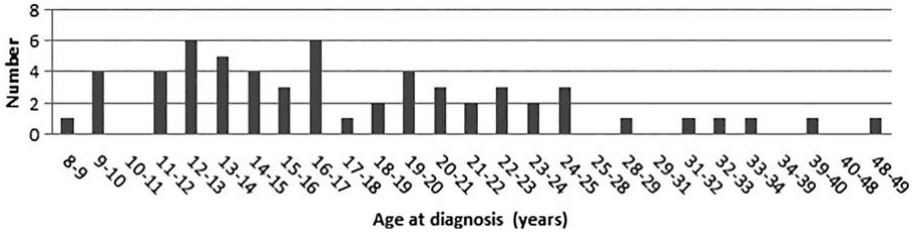

FIGURE 3. Age at diagnosis of colorectal cancer

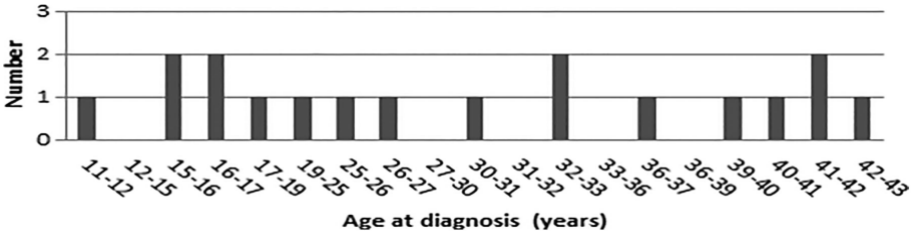

FIGURE 4. Age at diagnosis of small bowel cancer

CLINICAL DESCRIPTION AND NATURAL COURSE OF THE MOST COMMON CANCERS OBSERVED IN CMMRD

- **Brain Tumours**

  - **Glioblastoma**

Glioblastomas are tumours that originate from glia cells. According to the WHO grading system (2007), these tumours are classified as grade IV. The common symptoms and signs are related to raised intracranial pressure and include headache, nausea and/or vomiting and diplopia. Other symptoms include epileptic insults, unusual behaviour, and signs and symptoms caused by compression of parts of the brain and nerves.

The mean age at diagnosis in patients with CMMRD is 9 years (range 2–40 years) (Wimmer *et al*, in preparation). When a patient is diagnosed due to clinical symptoms, half of all patients already have advanced disease. The diagnosis is suspected with MRI or CT scanning and confirmed by histological examination of a biopsy. Glioblastoma are characterised by rapid progression, and the usual treatment consists of surgical resection (if possible), followed by radiotherapy and/or chemotherapy depending on the age of the child. Due to diffuse growth, complete resection of the tumour can be achieved in less than 50% of cases [7] and the reported 2-year survival in patients with sporadic glioblastoma is 30–50%.[7,8] The prognosis mainly depends on the completeness of the surgical resection. If the tumour is completely resected, a median survival of 106 months has been reported.[7] Glioblastoma is by far the most common cause of death in CMMRD, it is not known whether the natural course and response to treatment of glioblastoma in CMMRD differ from that of sporadic cases.

  - **Other brain tumours**

Other BTs reported in CMMRD include medulloblastoma and (S)PNET. Medulloblastomas are located in the cerebellum and (S)PNET in other parts of the brain. These tumours derive from primitive neuroectodermal cells and are classified as WHO grade IV tumours. The mean age at diagnosis in patients with CMMRD is 7 years (range 4–17 years). Frequently occurring signs and symptoms are related to increased intracranial pressure (nausea, headache, early morning

vomiting) and are usually already present for some time prior to diagnosis but go unrecognised. In the general population, the estimated median delay from the first symptoms to diagnosis is around 2 months.[9] At presentation, about 30% of tumours have metastasised via cerebrospinal fluid to other parts of the central nervous system. The diagnosis can be established using MRI and CT scanning. To evaluate whether the tumour has metastasised to the spinal cord, spinal MRI and a lumbar puncture are necessary. Treatment usually consists of surgical resection, radiotherapy (except in cases <3 years old) and chemotherapy. The prognosis is strongly dependent on the presence of metastases, and the prognosis of patients without metastases is relatively good (survival 75%) but neurocognitive consequences of brain radiotherapy may impair patient quality of life. Again, it is unknown whether the natural course of these tumours and the response to treatment in CMMRD differ from that of sporadic cases.

- **Digestive Tract Cancers: Colorectal Cancer and Small Bowel Cancer**
  - **CRC**

It is generally accepted that most sporadic and hereditary colorectal cancers originate from adenomas. This also appears to hold for patients with CMMRD, as colorectal adenomas are found in a third of all patients.[2]

Several studies have described digestive tract cancers in CMMRD cases. In the 29 CMMRD cases with CRC identified by Durno *et al*,[10] the mean age at diagnosis was 16.4 years (range 8–28 years), 30 years younger than the typical age at diagnosis of CRC in LS. Information on the presence of adenomas was provided for a total of 18 out of the 29 patients with CRC. All but one had at least one adenoma, and 10 (55%) had multiple adenomas with numbers usually between 10 and 100. In addition, 11 out of 29 patients with CRC (38%) had multiple CRCs. There was no predilection for a specific site in the colorectum.

Herkert *et al* described four patients with CMMRD with intestinal cancer and polyposis and biallelic *PMS2* mutations. In addition, these authors identified all *PMS2* CMMRD cases with gastrointestinal manifestations published in the literature between 1980 and December 2009.[11] They found 25 cases with gastrointestinal (small bowel and colorectal) polyps (mean age at diagnosis 17

years; range 7–46 years) and 42 cases with CRC (mean age at diagnosis 19; range 8–48 years). Full information on the colonic phenotype was available in 26 patients with CRC. Multiple adenomas (>10 adenomas) were found in 18 out of the 26 (70%) patients with CRC and multiple CRC in 38% of the cases.

Patients with CRC develop symptoms and signs, such as rectal blood loss, at a relatively late stage, and around 50% of the patients already have metastatic disease at diagnosis. The diagnosis of CRC is based on colonoscopy and confirmed by pathological examination of a tumour biopsy. The preferred treatment for CRC in young patients with LS is subtotal colectomy with ileorectal anastomosis (or proctocolectomy and ileal-pouch anal anastomosis in patients with rectal cancer). In patients with CMMRD with multiple polyps (if there are too many to remove endoscopically and/or if they show high grade dysplasia) and patients with CRC, the treatment of choice would be colectomy with ileorectal anastomosis or proctocolectomy and construction of an ileal pouch-anal anastomosis.

Patients with stage III CRC (and advanced stage II CRC) receive chemotherapy, and those with rectal cancer are treated with radiotherapy. No specific side effects of radiotherapy have been reported in LS. However, the effectiveness and toxicity of chemotherapy and radiotherapy in CMMRD is largely unknown (see below). Several studies have shown that there is an accelerated adenoma-carcinoma sequence in LS. Patients may develop CRC within 1–2 years after a normal colonoscopy. Our experience, supported by data from the literature on CMMRD, indicates that adenoma and CRC development are also characterised by rapid progression. The 5-year survival for CRC in LS is approximately 50–60%.

- **Small bowel cancer**

Cancer of the small bowel is also thought to originate from adenomas. Duodenal adenomas are found in 5% of CMMRD cases (Wimmer *et al*, in preparation). In a series of 42 primary SBCs in LS, the mean age at diagnosis was 49 years (range 25–88 years), about 10–15 years younger than in sporadic SBC.[12] Based on 18 SBCs in 12 patients, the mean age at diagnosis in CMMRD is 25 years (11–42 years) (Wimmer *et al*, in preparation).

Herkert *et al*[11] identified 25 cases with gastrointestinal (small bowel and colorectal) polyps (mean age at diagnosis 17 years; range 7–46 years) in the medical literature, and 11 with SBC (mean age at diagnosis 27; range 11–42 years).

About 50% of sporadic SBCs are located in the duodenum and 10–15% in the ileum, whereas in LS the cancers are more evenly distributed along the small bowel.[12] The 11 *PMS2* CMMRD literature cases identified by Herkert *et al*[11] developed 18 SBCs, including 8 duodenal cancers, 7 jejunal cancers and 3 ileal cancers. Three of the 11 patients developed multiple (up to 5) SBCs.[5]

In general, SBCs go unnoticed for a long period or manifest with only non-specific symptoms such as dull, cramping abdominal pain, abdominal distention and (faecal occult) blood loss. Obstruction is also a common presentation.

The diagnostic modalities used for assessing the presence of SBC are radiographic imaging (CT or MRI enteroclysis) and endoscopy (upper gastrointestinal (GI) endoscopy and ileocolonoscopy, for the detection of cancers located in the duodenum and ileum, respectively). There is also an increasing use of video capsule endoscopy (VCE) and double balloon enteroscopy. The effects of chemotherapy and radiotherapy are disappointing,[13] and the treatment of choice is surgical resection. The 5-year survival rate of patients with resected tumours is around 50%. The natural history and prognosis of patients with CMMRD with SBC are unknown.

- **Haematological Cancers**
  - **Non-Hodgkin lymphoma**

NHL is the most commonly occurring haematological cancer in CMMRD (Wimmer *et al*, in preparation), with T cell NHL more frequently observed than B cell NHL. T cell NHL is usually located in the mediastinum, while B cell NHL has a mainly intra-abdominal location but is sometimes seen in the cervical region. Signs and symptoms vary depending on the type of lymphoma and the location, but may include coughing and respiratory distress (T cell NHL), obstruction of the bowel (B cell NHL), cervical lymphadenopathy, difficulty with swallowing (also

seen in B cell NHL), and anaemia, tiredness and bruises in cases with bone marrow involvement.

Ages at diagnosis of sporadic NHL vary from 5 years to 12 years, according to histological subtypes.[14] The median age at diagnosis of NHL in CMMRD is 5 years (range 0.4–17 years), based on 31 cases (Wimmer *et al*, in preparation). In the general population, the median time to diagnosis, defined as the interval between the first signs and symptoms and diagnosis, is relatively brief (3.8 weeks).[9] The techniques used to diagnose and stage NHL may include ultrasound of the abdomen, lymph nodes and testes, MRI or CT scanning, fluorodeoxyglucose-positron emission tomography (FDG-PET) scanning, biopsy of the tumour, bone marrow biopsy and lumbar puncture. Treatment for NHL consists mainly of intensive chemotherapy, with radiotherapy restricted to the small percentage of patients with overt central nervous system (CNS) disease at the time of diagnosis. The duration of treatment varies from a few weeks to 2 years, depending on the stage and the histological subtype. The prognosis is relatively good, with survival rates of 70–90%. The natural course and response to treatment of NHL associated with CMMRD is unknown.

- **Acute leukaemia**

The most common form of acute leukaemia in CMMRD is acute lymphoblastic leukaemia (ALL). The mean age at diagnosis of ALL in CMMRD is 6 years (range 2–21 years), based on nine cases (Wimmer *et al*, in preparation). The incidence of ALL peaks between 2 years and 5 years in non-CMMRD. Children with ALL often present with signs and symptoms that reflect bone marrow infiltration and/or extramedullary disease including anaemia, thrombocytopenia, neutropenia and lymphadenopathy. Other presenting signs and symptoms are bone pain, fever, fatigue, bleeding and respiratory distress. The median time from the presentation of signs and symptoms to diagnosis is only 1–2 weeks.[9] Tests required to classify ALL include immunotyping, cytogenetic studies and molecular studies to identify translocations. Lumbar puncture is performed to assess the involvement of the CNS. The diagnosis of ALL is confirmed by a bone marrow aspiration and biopsy. Although the treatment of ALL is primarily based

on chemotherapy, the different forms of ALL require different approaches for optimal results. The prognosis of ALL depends on the clinical and laboratory features and the response to treatment. Overall, the cure rate of patients without CMMRD with ALL is greater than 80%.

CONSIDERATIONS REGARDING THE SURVEILLANCE PROGRAMME IN PATIENTS WITH CMMRD

- **Brain Tumours**

MRI scanning is the best screening method for the early detection of BT. Repeated CT scanning of the brain should be avoided because of the possible induction of tumours due to radiation.

As previously mentioned, glioblastomas usually show diffuse growth, meaning that discrimination between normal and tumour tissue may be impossible and the precise extent of the tumour may be difficult to assess. MRI scanning in young children is usually performed under general anaesthesia. MRI starting from birth is recommended by Durno *et al.*[4] The youngest patients with CMMRD diagnosed with glioblastoma were 2 years old. Therefore, we recommend commencing MRI scanning at the age of 2 years, and due to rapid progression, scanning at an interval of 6–12 months is probably needed. Whether early detection will lead to more complete surgical resections and improved survival is presently unknown.

- **Digestive Tract Cancer: CRC**

Many studies have demonstrated that colonoscopy has the highest sensitivity and specificity and is thus the best tool for surveillance of the colon. In LS, small, flat and non-polypoid lesions are frequent and can easily be missed.[15,16] We also observed mainly multiple non-polypoid lesions in a patient with CMMRD. Therefore, it is recommended that chromoscopy be used in order to allow the detection and delineation of small, flat lesions. In children, colonoscopy is performed under general anaesthesia.

Based on experience with LS, surveillance of the colon is expected to be effective. Due to the assumed high progression rate from an adenoma to colorectal cancer,

an intensive surveillance programme at annual intervals is probably needed. In patients with multiple adenomas, a shorter interval of 6 months is recommended. Because most cancers develop in the second decade of life, the programme can be started by the age of 8 years. In view of the large non-polypoid lesions often observed in CMMRD, a paediatric gastroenterologist should perform the procedure together with an 'adult' gastroenterologist with experience of endoscopic mucosal resection of such tumours.

- **Digestive Tract Cancer: Small Bowel Cancer**

For the detection of duodenal cancers, an upper GI endoscopy can be performed (at the same time as colonoscopy). During colonoscopy, the terminal ileum should also be intubated for the identification of ileal cancer. CT scanning and MRI enteroclysis can be used for the detection of SBC located in the jejunum and remaining ileum but these modalities are too burdensome for surveillance purposes. A major disadvantage of regular CT scanning is the radiation burden. VCE is probably the best tool. Two studies in LS have shown that adenomas and SBC can be detected using VCE. In a French study by Saurin, tumours were detected in 10% of cases (one jejunal cancer, two adenomas).[17] However, in a Dutch study on 200 LS mutation carriers, a tumour (one adenoma and one cancer) was identified in only 1.5% of cases (Haanstra *et al* submitted 2013). In this study, one patient developed a SBC 6 months after a normal VCE. The value of surveillance of the small bowel using VCE is therefore unknown. However, the high prevalence of such tumours (8%) in CMMD-R may justify the use of VCE. Because SBC below age 10 years is very rare, the surveillance programme can be started at the age of 10 years. Young children are generally able to swallow the capsule. An alternative is to place the capsule endoscopically during upper GI endoscopy.[11]

- **Non-Hodgkin Lymphoma**

Because sporadic T cell and B cell lymphomas have an excellent outcome, the benefit of early diagnosis is not obvious except for the avoidance of life-threatening situations at diagnosis for patients with huge mediastinal masses.

We currently have no information on the natural history of these lymphomas in CMMRD, and while the natural course of the disease might differ from sporadic cases, sporadic cases usually present with rapidly growing tumours and clinical manifestations within a month prior to diagnosis. Thus, screening at less than 3-month intervals would probably be inefficient, whereas this frequency is probably too high for a screening for which we are not sure that it would improve the cure rate. A reasonable alternative is to perform clinical examinations, and optionally, abdominal ultrasound every 6 months. This strategy would probably be inefficient for early diagnosis but it might provide useful information on the natural history of this lymphoma in patients with CMMRD.

- **Acute Lymphocytic Leukaemia/Acute Myeloid Leukaemia**

Signs and symptoms of acute leukaemia are apparent within a short period of time. As most patients with ALL have anaemia, thrombocytopenia with a normal or depressed white blood cells and lymphoblasts on peripheral smear, regular assessment of the blood count may be recommended but it is uncertain whether surveillance will lead to early detection and improvement of the prognosis.

CHEMOTHERAPY IN LYNCH SYNDROME AND CMMRD

About 15% of CRCs shows microsatellite instability (MSI), which is a marker of MMR deficiency. In sporadic CRC, MSI results from somatic MMR inactivation mainly due to epigenetic changes in the tumour, whereas in LS, MSI is caused by biallelic MMR gene inactivation due to a heterozygous germ line mutation and a somatic second-hit alteration in the other allele. Patients with CMMR-D show MSI due to a constitutional MMR defect caused by biallelic germ line MMR gene mutations.

A number of studies have evaluated the effectiveness of chemotherapy in the subgroup of CRC with MMR deficient versus MMR proficient tumours. Chemotherapy is the mainstay of treatment of many cancers in childhood. Due to the rarity of CMMR-D, very limited information on the optimal chemotherapy is available. Several studies have demonstrated that tumours with loss of MMR function are more frequently resistant to certain forms of chemo-

therapy.[18] Another major concern is that some of these chemotherapeutic agents are mutagenic and may increase the risk of developing therapy-induced cancers, because patients with CMMR-D have a defect in DNA damage signalling and are unable to repair the accumulated somatic mutations.[19]

The effectiveness of chemotherapy in MMR-deficient CRC has recently been reviewed by Devaud and Gallinger.[20] Evaluation of clinical trials performed in the 20th century that compared fluorouracil (5FU) with no treatment [21,22] demonstrated that MSI-high (MSI-H) tumours (retrospectively analysed) were resistant to treatment with 5FU. These observations were confirmed by many in vitro studies.[23] The resistance to 5FU of MMR-deficient cancer cells may be due to the incorporation of fluorouracil metabolites into DNA, although the sensitivity of MMR-deficient cell lines to this drug and other agents is likely to be dependent on the status of HSP110, a molecular chaperone that has been reported to be mutated in MMR-deficient tumours, sensitising MSI-tumour cells to a wide spectrum of anticancer agents including 5-FU[24] (Collura A, *Gastroenterology*, in press).

Although studies of MMR-deficient cell lines reported resistance to cisplatin and carboplatin, a good response to oxaliplatin was found and this was subsequently confirmed in clinical trials.[13, 25–28] Irinotecan also appears to be effective, similarly as in MMR-proficient tumours.[29–32] Moreover, a recent study using cell lines showed a good response to irinotecan in combination with thymidine.[33]

Most of the above-mentioned studies were performed in patients with somatic deficient MMR tumours. Less is known about the response in patients with LS and patients with CMMR-D with CRC.

All patients with CMMR-D with CRC and treated with chemotherapy described in the literature are shown in table 3.[34–43] Most patients appear to show a response similar to the response in patients with sporadic CRC. However, prospective studies are needed to confirm this observation.

In 2007, Scott *et al*[44] discussed the effectiveness of chemotherapy in patients with CMMR-D. Several cell line and mouse model studies showed that tumours are resistant to treatment with O6-methylating agents.[18] One of these agents (temozolomide) is frequently used in the standard treatment of glioblastoma.

This drug causes mutations in tumour DNA that cannot be repaired by patients with a loss of MMR function. Indeed, investigation of a clinical specimen from a patient treated with this drug showed an accumulation of somatic mutations (mutator phenotype).[45] In vitro studies showed a similar effect for busulfan but not for chloroethylating agents such as cyclophosphamide and melphalan.[18]

MSI occurs in some tumours following therapy with thiopurines or cisplatin, suggesting that MMR deficiency is important in clinical resistance.[46–48] In addition, MMR deficiency appears to be common in resistant acute myeloid leukemia and is a recurrent feature of secondary NHL occurring in allografted patients treated with azathioprine.[49–51] A report on two patients with glioblastoma showed that they were resistant to treatment with temozolomide.[52] Another study demonstrated loss of *MSH6* expression in a subset of patients with glioblastoma resistant to temozolomide.[53]

All patients with CMMR-D known from literature with BT [35, 36, 38, 43 44, 52, 54–58] and lymphoma [38, 52, 57, 59–63] that were treated with chemotherapy are listed in tables 4 and 5. Most patients with T cell lymphomas showed a good response to chemotherapy. However, chemotherapy in patients with BT had a less favourable outcome. In particular, only one out of six patients treated with temozolomide and radiotherapy showed a partial response. In the other patients, the tumour was resistant to treatment.

Whether temozolomide or other drugs such as cisplatin and busulfan are contraindicated in CMMR-D is currently controversial and requires further studies. As stated by Scott *et al*,[44] early detection of tumours may allow considerations about the most effective chemotherapeutic regimen.

TABLE 3. Response of colorectal cancer (CRC) to chemotherapy in CMMRD

Author/ Year	*Type of tumour*	*Gene defect*	*Age (yrs)*	*Surgery*	*Chemotherapy*	*Radio-therapy*	*Response*	*Status*
Gallinger/ 2004	3x CRC	*MLH1*	9	Subtotal colectomy (3 polyps)	irinotecan, 5FU, leucovorin	No	No evidence recurrence	After 11 months alive
Gururan-gan/2007	CRC sigmoid	*PMS2*	14	Pancolectomy	5FU, leucovorin	Yes	No evidence recurrence	Died 7 years later from brain tumour
Tan/2008	CRC	*PMS2*	15	Low anterior resection	FOLFOX4/12x	No		
				Panproctocolectomy (3× CRC, >10 polyps in specimen)			Not reported	Not reported
Jackson/ 2008	CRC sigmoid Liver metastases	*PMS2*	14	Resection sigmoid, partial liver resection; colectomy	Oxaliplatin, capacitabine			5 months later recurrence rectal cancer
			14	Resection rectal cancer, RFA liver metastases	irinotecan, 5FU, bevacizumab		Not reported	Alive
Kruger/ 2008	Sigmoid CRC	*PMS2*	13	Sigmoid resection, later panproctocolectomy (multiple polyps, CRC right colon)	FOLFOX/ 10 months	No	No evidence recurrence	Alive
Rahner/ 2008	4x CRC	*MSH6*	17	Colectomy	Cetuximab	No		Not reported
Kratz/ 2009	CRC	*PMS2*	8	Transversectomy	FOLFOX			Alive
Toledano/ 2009	Rectal cancer, 20 polyps	*MSH2*	14	Panproctocolectomy (100 polyps)	FOLFOX	Yes		Relapse after 2 months
					FOLFIRI/ avastin			Died 12 months after diagnosis from brain tumour

Vasovcak /2012	Rectal cancer	*PMS2*	13	Resection	Capecitabine, oxaliplatin, followed by cetuximab, irinotecan			Resistant after 4 cycles
						Yes		Died after short remission 17 months later
Lindsay/ 2013	CRC, liver metastases	*PMS2*	12	left hemicolectomy	Folinic acid, 5FU, oxaliplatin; later irinotecan, 5FU, Leucovorin	No	Partial remission	2 years later new liver metastases; died from progressive leukoencephalopathy

5FU, fluorouracil; CMMR-D, constitutional mismatch repair-deficiency; CRC, colorectal cancer; FOLFIRI, folinic acid, fluorouracil and irinotecan; FOLFOX, folinic acid,fluorouracil and oxaliplatin; RFA, radio frequency ablation.

DISCUSSION

For many types of cancers, diagnosis at the earliest possible (preferably preclinical) stage results in much more effective treatment and an improved prognosis. This may also be the case for CMMR-D. Based on the available knowledge and the discussions at the workshop, the European consortium proposed a surveillance protocol as summarised in table 6. The surveillance for CRC is the only aspect that largely complies with the WHO criteria for screening, although it is unknown whether colonoscopic surveillance in CMMR-D is as effective as in LS. The value of all other suggested screening protocols is unknown. In particular, it is questionable whether surveillance for ALL and NHL improves the already favourable outcome for patients with these tumours. A randomised controlled trial is needed to assess whether surveillance can improve the prognosis. However, the question then arises whether it is ethically justified not to offer surveillance in view of the high mortality without surveillance. As most parents/patients would probably choose to participate in a surveillance programme, performing a trial would be difficult. The best approach may be to discuss the advantages and disadvantages of surveillance and to make a joint decision (table 7).

A general recommendation is that parents (or adult patients) should contact their doctor if the child (adult patient) develops unusual signs or symptoms. This is especially important in the case of BT, in view of the rather long time to diagnosis.

The programme recommended by the consortium aims to detect the most common cancers. However, a variety of other tumours also occur in CMMR-D (table 2). A prospective randomised trial may be performed to test whether a once yearly (rapid) whole body MRI adds some efficacy to our screening programme.

As already mentioned above, it should be emphasised that the distribution of ages at diagnosis of the various cancers is biased by collection of published cases. In fact, ascertainment bias may be a major issue in all that we know about this condition. As further study is done, less severe cases may possible be seen more frequently.

Nevertheless, the high risk of developing a wide variety of cancers, the high risk of developing multiple cancers, sometimes synchronously, the occurrence of these cancers at a young age and the need for intensive multimodal treatment in the case of cancer, imposes an enormous burden on parents and patients. A recent evaluation of psychological distress in individuals predisposed to develop cancer showed increased levels of distress in adults at risk of developing multiple tumours.[64] Nothing is known about the psychological distress in patients with CMMR-D and their parents. Therefore, doctors involved in the care of these patients should offer the support of a psychologist.[1] In addition, the clinical geneticist plays an important role by organising pre-symptomatic testing of other family members for CMMR-D or LS, and through discussion of the option of prenatal or pre-implementation genetic diagnosis.

Individuals at risk for multiple cancers usually see several doctors including paediatric oncologists, neurologists, (neuro)surgeons, gastroenterologists and haematologists. It is important that one of these specialists coordinates the surveillance examinations and is available for the patients if they have questions.

A prerequisite for participation in a surveillance programme is prospective collection of all results of surveillance, including the response to treatment. With this aim in mind, the European consortium established an EU-CMMR-D database. This European registry will allow the (cost)effectiveness of the surveillance programme to be evaluated and will allow the sensitivity of these tumours to chemotherapy to be assessed.

TABLE 4. Response of brain tumours (BTs) to chemotherapy in CMMRD

Author/ Year	*Brain tumour. (gene defect)*	*Age (years)*	*Surgery*	*Chemotherapy*	*Radio-therapy*	Response	Outcome	Status
Menko/ 2004	Oligodendroma *(MSH6)*	10	Partial resection	Not specified	Yes	Partial remission	Recurrence of BT	Died at 12 years from BT
Gurugan-gan/2007	Astrocytoma grade III *(PMS2)*	19	Subtotal resection	Temozolonide after RT	Yes	Resistant	New BT	
		19		Carmustine and irinotecan		"Very good" partial remission	18 months later minimal uptake scan	Died 22 months later from progression BT
Scott/ 2007	Medulloblastoma (*MSH6*)	7	Not specified	SIOP PNET 3: vincristine, cyclophosphamide etoposide, carboplatin	Yes spinal cord	Complete response		Alive at 13 years
Tan/ 2008	Glioblastoma *(PMS2)*	8	No	Temozolomide later: temozolomide and lomustin	Yes	Resistant	Leptomeningeal disease	Died 10 months after diagnosis
Etzler/ 2008	Medulloblastoma (*MSH6)*	6	Gross total resection	Cisplatin CCNU vincristine	Yes spinal cord	Not reported	MDS/AML at 9	Died 36 months later
Kruger/ 2008	Glioblastoma (*PMS2*)	6	Surgical resection	Yes not specified	Yes	complete remission	Recurrent BT 10 years later	Died at age 16 years from relapse of BT
Kruger/ 2008	Glioblastoma (*PMS2*)	6	Surgical resection	Yes not specified	Yes	Resistant	Unresectable tumour	Died at age 7 years
Ilencikova /2011	Fibrillar astrocytoma (*MSH6*)	11	No	POG 9233/34	Yes	Resistant	< 1 year new tumour, anaplastic astrocytoma III	
		11		HIT-AGG-2007 plus temozolomide	Yes	Resistant		Died 3 months later
Ilencikova / 2011	Glioblastoma (*MSH6*)	10	Surgical resection	Temozolomide	Yes	Resistant	Progression	Died at age 11 years

Johann-esma/ 2011	Cystic glioma (*PMS2*)	11	Resection	No	No		Recurrence after 7 months	
		11	Debulking	Vincristine, carboplatin, VP16, etoposide	Yes	Partial response	New tumour after 2 years, 8 months, high grade glioma	
		14		Carboplatin, 95elphalan, bone marrow transplantation	Yes	Partial response	Recurrence 3 years, 4 months later	
		18		Temozolomide	Yes	Resistant		Died at age 19 years
Baas/2013	Glioblastoma (*PMS2*)	3	Surgical resection	Temozolomide	Yes	Partial response; “26 months healthy		Died at 5 years 6 months due to sepsis during chemotherapy for NHL
Baas/2013	Anaplastic astrocytoma/ spinal cord (*MLH1*)	2 years 10 months	Surgical resection	HIT-GBM-D protocol	Yes	Partial response		Died at 5 years 6 months due to sepsis during chemotherapy for NHL
Lindsay/ 2013	Medulloblastoma & metastatic CRC (*PMS2*)	12	Complete resection	folinic acid, 5FU, oxaplatin for CRC	Yes craniospinal	Complete response		Died at 6 years and 3 months due to sepsis
Yeung/ 2013	Optic pathway glioma (*PMS2*)	3	No	Carboplatin, vincristine		partial response	GH-excess	Stable disease during 4½ years
		8		Vinblastine followed by temozolomide		Resistant		Increased activity scan, alive

5FU, fluorouracil; AML, acute myeloid leukemia; CCNU, Lomustine; CMMR-D, constitutional mismatch repair-deficiency; CRC, colorectal cancer; GH, growth hormone; NHL, non-Hodgkin lymphoma, PNET, primitive neuroectodermal tumours.

TABLE 5. Response of NHL to chemotherapy in CMMRD

Author/ Year	*Type of lymphoma*	*Gene defect*	*Age (yrs)*	*Chemotherapy*	*Other therapy*	*Response*	*Outcome/ Status*
Ostergaard/ 2005	T cell NHL	*MSH6*	10	Yes	No	Partial remission	Died from progression 6 months later
Scott/2007	T cell NHL	*MSH2*	2.5	BFM-NHL95 & MRC ALL 97/99	No	Partial remission	Recurrence 2 weeks after completion of chemotherapy
			2.5	MRC ALL R3 protocol (minus cyclophosphamide) etoposide	Total body RT, peripheral blood stem cell transplantation	Complete remission	Alive, 6 years
Kratz/2008	T cell NHL	*PMS2*	6	BFM-NHL95	No	Complete remission	Alive, 16 years
Kruger/ 2008	T cell NHL	*PMS2*	10	Euro-LB 02	No	Complete remission	Alive
Peters/ 2009	T cell NHL	*MSH6*	8	Children's Oncology group protocol A 5971	No	Complete remission	Relapse 9 months after diagnosis
			8-9	ifosfamide, cisplatin, etoposide, nelarabine	Radiotherapy	Resistant	Progression, died at age 9 years
Ripperger/ 2010	T cell NHL	*MSH6*	6	BFM-NHL95	No	Not specified	Relapse 6 months after completion of chemotherapy
				ALL REZ BFM 2002	Haematopoietic stem cell transplantation	Complete remission	Alive 7 years later
Ilencikova/2 011	T cell NHL	*MSH6*	11	Euro-LB 2002		Good response	died from BT <1yr
Baas/2013	T cell NHL	*MLH1*	5.5	Children's Oncology Group-A5971	No		died from staphylococc. Sepsis
Baas/2013	B cell NHL	*PMS2*	9	SNWLK-94	No	Complete remission	11years, 6 months: T cell NHL
				Euro LB-02 protocol	No	Not reported	
Baas/2013	T cell NHL	*PMS2*	5.5	Euro LB-02	No	"initially good response"	Died 9 months later from sepsis during bone marrow aplasia

ALL, acute lymphoblastic leukaemia; CMMR-D, constitutional mismatch repair-deficiency; NHL, non-Hodgkin lymphoma.

cONCLUSION

In an era of high-throughput sequencing technologies, an increasing number of patients with CMMRD are being identified. Most patients die from cancers in early childhood. The only way to improve the prognosis is surveillance for these cancers. Surveillance for CRC, and possibly SBC, might be effective; however, the value of surveillance and early detection of BT and NHL/leukaemia is unknown. The best approach is to discuss the advantages and disadvantages of surveillance. When patients and parents decide to participate, outcomes should be collected and evaluated within the EU registry.

TABLE 6. Surveillance protocol for CMMRD patients proposed by the European Consortium

Type of cancer	*Lower age limit*	*Procedure/interval*
Brain tumours	from age 2 years	MRI, 1x/6-12 months
Digestive Tract Cancers		
SBC	from age 10 years	VCE, upper gastrointestinal endoscopy*; 1×/year
CRC	from age 8 years	Ileocolonoscopy; 1×/year;
Haematological Malignancies		
NHL/Other Lymphoma	from age 1 year	Clinical examination 1×/6 months Optional: abdominal ultrasound 1×/6 months
Leukaemia	from age 1 year	Blood count 1x/6 months
LS-associated Cancers †	from age 20 years	Gynaecological examination, transvaginal US, Pipelle curettage (1×/year), Urine cytology, dipstick (1×/year)
All Cancers	Parents and patients should be advised to contact their doctor in case of unusual signs or symptoms. A pamphlet should be available with information about the signs/symptoms that may occur.	

*At the same time as colonoscopy under general anaesthesia.

†See: Revised guidelines for the clinical management of Lynch syndrome: HFA Vasen *et al Gut* 2013.

CMMRD, constitutional mismatch repair-deficiency; CRC, colorectal cancer; LS, Lynch syndrome; SBC, small bowel cancer; NHL, non-Hodgkin lymphoma; VCE, video capsule endoscopy.

TABLE 7. Pros and cons of participating in a surveillance program

Pros	*Cons*
1. Possible early detection of BTs, allowing complete resection 2. Early detection of adenomas in the small bowel and colorectum before malignant degeneration 3. Early detection of BTs, which allows the most effective surgery and chemotherapeutic regimen to be determined 4. Early detection of NHL and leukaemia, which allows the most effective chemotherapeutic regimen to be determined	1. Brain MRI may reveal small lesions of unknown significance. The only management is follow-up MRI at a short interval, which may increase anxiety 2. Claustrophobia (MRI) in adults 3. Video capsule endoscopy: risk of retaining capsule 4. Colonoscopy: risk of perforation (1/1000) and risk of bleeding after polypectomy (3–4%) 5. Greater awareness of being at high risk for developing cancers 6. Increased psychological distress before and after surveillance examination 7. Uncertainty about effectiveness of prevention programme and early treatment 8. Complications of general anaesthesia

- BT, brain tumour; NHL, non-Hodgkin lymphoma.

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CHAPTER 6

High Yield of Surveillance in Patients Diagnosed with Constitutional Mismatch Repair Deficiency

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ABSTRACT

Background: Constitutional Mismatch Repair Deficiency (CMMRD) is a rare autosomal recessively inherited syndrome that is caused by bi-allelic pathogenic variants of the mismatch repair genes. It is characterized by the development of multiple tumors in the first and second decade of life including brain, gastrointestinal and hematological tumors often resulting in early death. In order to improve the prognosis of these patients, the European collaborative group "Care for CMMRD" (C4CMMRD) developed a surveillance program in 2014 and established a registry of CMMRD patients in Paris. The aim of the study was to evaluate the outcome of this program.

Methods: Twenty-two patients with a definitive diagnosis of CMMRD and with at least one follow-up study were selected from the registry. Medical data on the outcome of surveillance were collected from these patients.

Results: During a mean follow-up of four years, the program detected eight malignant tumors including three brain tumors, three upper gastrointestinal cancers, and two colorectal cancers. Most tumors could successfully be treated. In addition, many adenomas were detected in the duodenum, and colorectum and subsequently removed. Seven patients developed a symptomatic malignancy, including two brain tumors, one small bowel cancer, and four hematological malignancies. At the end of the follow-up, 16 out of 22 patients (73%) who participated in the surveillance program were still alive.

Conclusion: The study suggests a beneficial effect of surveillance of the digestive tract and brains.

INTRODUCTION

One of the most frequent inherited forms of cancer is Lynch syndrome (LS), which is characterised by high risks of developing colorectal cancer (CRC), endometrial cancer and other cancers. LS is an autosomal dominant inherited syndrome caused by pathogenic monoallelic variants in the mismatch repair (MMR) genes including *MLH1, MSH2, MSH6* and *PMS2*.[1] Biallelic pathogenic germline variants of the MMR genes result in a very rare syndrome usually referred to as constitutional mismatch repair deficiency (CMMRD). CMMRD is an autosomal recessive syndrome characterised by multiple cancers that develop in childhood including brain tumours, cancers of the digestive tract, haematological malignancies and other tumours.[2]

In CMMRD, brain tumours and haematological malignancies are often diagnosed in the first decade of life. If the patients survive these cancers, they may develop cancers of the digestive tract including CRC, small bowel cancer (SBC) and other tumours associated with LS in the second decade of life or later.[2 3] One of the most striking features is the very high risk of developing multiple tumours, synchronously or metachronously.

The life expectancy of patients with CMMRD is very limited as many patients will die in the first or second decade of life frequently due to brain tumours. Early detection and treatment is the only way to improve the prognosis. Identification of CMMRD is therefore of utmost importance because it allows implementation of preventative strategies including genetic counselling of parents and tumour surveillance for the patient.

Currently, various guidelines are available that can be used to guide the management of these patients.[4–7] In 2012, Durno *et al* reported the successful outcome of a surveillance protocol for the first time implemented in a kindred with CMMRD.[4] Two years later, the European collaborative group (C4CMMRD), collected data on the natural history of the tumours involved in this syndrome and developed a new protocol using this information.[2 5] In 2017, the US Multi-Society Task Force on CRC with invited experts developed a consensus statement and recommendations for the management of patients with CMMRD.[7] The aims of the present study are (1) to assess the effectiveness of the C4CMMRD

surveillance programme and (2) to discuss possible improvements of the protocol.

METHODS

At the meeting of the C4CMMRD group in 2014, the collaborative group decided to set up an European Registry of patients with CMMRD to enable various research projects. One of the purposes of the registry was to collect prospective data to better understand the natural history of the disease; another purpose was to investigate the effectiveness of surveillance in patients that underwent periodic examination. Only patients with a definitive diagnosis of CMMRD are registered. The registry, based in Paris, includes medical data, family history of cancer, previous malignancies, genetic tests results and outcome. All data are pseudonymised.

For the present study, patients were selected from the Paris registry who underwent at least one surveillance examination. Anonymous medical data were retrospectively collected. The observation time is between the first examination and the last screening examination or date of death.

RESULTS

Basic characteristics

A total of 22 patients (11 females and 11 males) were included in the study. The most common underlying biallelic pathogenic variants were in *PMS2*, detected in 15 patients, followed by biallelic *MSH6* pathogenic variants in 4 patients and biallelic *MSH2* pathogenic variants in 3 patients. Eighteen of the 22 patients had developed 27 malignancies before start of the surveillance programme including 12 CRCs, 8 lymphomas, 2 leukaemia, 4 brain tumours and 1 patient was diagnosed with a pilomatricial carcinoma. The characteristics of the patients are summarised in table 1.

TABLE 1. Detailed information of the study group of CMMRD patients

Pt No.	*Sex*	*Gene*	*Age @ first screening (years)*	*Previous cancers (Age, if known)*
1	M	*PMS2*	10	Lymphoma
2	F	*PMS2*	7	-
3	M	*PMS2*	27	-
4	F	*PMS2*	21	Two synchronous CRC (19)
5	F	*MSH2*	22	CRC (22)
6	M	*PMS2*	14	Lymphoma (8), Lymphoma (14)
7	F	*PMS2*	17	Two synchronous CRC (17)
8	F	*PMS2*	12	BT- glioblastoma (12)
9	F	*MSH6*	7	-
10	M	*PMS2*	8	Lymphoma
11	M	*MSH6*	9	Leukemia (3), Lymphoma (7)
12	M	*MSH2*	12	CRC (12)
13	M	*PMS2*	5	Lymphoma (3)
14	M	*MSH2*	24	CRC (23)
15	F	*PMS2*	18	BT- Glioblastoma (18)
16	M	*PMS2*	13	Leukemia, Pilomatrix carcinoma, CRC
17	F	*MSH6*	10	BT- Glioblastoma (9)
18	M	*PMS2*	17	Lymphoma, CRC (17)
19	F	*MSH6*	7	CRC (7)
20	F	*PMS2*	27	Two synchronous CRC, Lymphoma
21	M	*PMS2*	12	-
22	F	*PMS2*	17	BT-Medulloblastoma (4)

BT, brain tumour; CMMRD, constitutional mismatch repair deficiency; CRC, colorectal cancer

TABLE 2: Characteristics and management of cancers detected during the screening program

Site	*Type of Tumor (Stage)*	*Mode of diagnosis*	*Age at dx (yrs)*	*Treatment/Outcome*
Brain	Glioblastoma	Symptomatic	8	Surgery, radiotherapy & chemotherapy; Local recurrence after 11 months; Deceased due to liver failure after 16 months.
	Glioblastoma	Symptomatic	31	Surgery, radiotherapy & chemotherapy; Deceased after 2 years due to complications of the tumor.
	Astrocytoma (grade 3)	Screen-detected	8	Surgery and radiotherapy; Alive at his last follow-up, 3.5 years after the diagnosis.
	Anaplastic Oligodendro-glioma (grade 3)	Screen-detected	11	Surgery and radiotherapy; Alive at her last follow-up, 2 years after the diagnosis.
	Glioblastoma	Screen-detected	19	Right frontal resection; Local recurrence one month after the initial diagnosis. Deceased due to increased cerebral pressure.
Upper GI	Gastric Cancer (T3N1)	At first screening	23	Neo-adjuvant chemotherapy and surgery; Alive 3 years after diagnosis with no abnormalities detected on the follow-up endoscopies.
	Gastric Cancer	Screen-detected	10	Future treatment refused because of advanced metastatic disease CRC; The patient died less than 2 months after gastric cancer diagnosis

	Esophageal Cancer (T1aN0)	At first screening	18	Neo-adjuvant chemotherapy and surgery; The patient died after 16 months due to the complications of glioblastoma.
Small Bowel	Small Bowel Cancer (T3N2M1)	Symptomatic	26	Palliative chemotherapy Alive 4 years after diagnosis
Colorectum	Pouch Adenocarcinoma (T2N2)	Screen-detected	28	Pouch resection; no adjuvant treatment. Alive 27 months after diagnosis
	Colorectal Carcinoma (T1N0)	Screen-detected	19	Endoscopic resection; additional subtotal colectomy; No residual malignancy in colectomy specimen. The patient died after 10 months due to the complications of glioblastoma.
Hematolog-ical	Myelodysplastic Syndrome	Symptomatic	15	Pt deceased shortly after diagnosis
	T-Cell Lymphoma (Ann Arbor Stage IV)	Symptomatic	8	Conventional chemotherapy Mediastinal & testicular CR ; persistent MRD
	Acute Lymphoblastic Leukemia	Symptomatic	12	no additional information
	Acute Lymphoblastic Leukemia	Symptomatic	14	Chemotherapy plus allogenic HSCT Complete remission

CR, complete remission; CRC, colorectal cancer; GI, gastrointestinal; HSCT, haematopoietic stem cell transplantation; MRD, minimal residual disease.

Outcome of surveillance

The median age at the first screening examination was 13.2 years (range: 5.9–27.6). The mean follow-up time between the first screening examination until the last follow-up was 48.2 months (SD=21.8). Six (27%) of the 22 patients died during follow-up. Causes of death included brain tumour in three patients, myelodysplastic syndrome in one patient, metastatic GI tumour in another patient and liver failure in a patient diagnosed with local recurrence of glioblastoma. The outcome of surveillance is summarised in table 2.

Surveillance for brain tumours

The programme recommended by the C4CMMRD group includes an MRI at intervals of 6–12 months starting from the age of 2 years. All patients except one patient who refused screening of the brain, underwent biannual or annual MRI.

A total of five brain tumours were diagnosed during follow-up of which three were detected by screening, one tumour was a symptomatic interval cancer and one patient who refused MRI surveillance developed a symptomatic brain tumour. In addition, one other patient was found to have a suspicious finding on his last screening MRI which was under diagnostic workup.

A glioblastoma was detected in a woman aged 19 years while she was under yearly MRI surveillance. She underwent a right frontal resection. Already 1 month after treatment, she developed a local recurrence. Shortly thereafter, the patient died due to increased cerebral pressure. Seven years earlier, the patient had been treated for another primary brain tumour.

An anaplastic oligodendroglioma (grade 3) was detected by MRI screening in a girl aged 11 years. The patient was undergoing biannual MRI screening and there were few intracerebral hyperintense foci without postcontrast enhancement or diffusion restriction which were stable in the previous 4 years of screening. The patient underwent block-resection and radiotherapy and she was alive at her last follow-up, 2 years after the diagnosis.

An astrocytoma (grade 3) was detected in a boy aged 8 years, detected with screening 5.5 months after the previous normal MRI. The patient underwent complete resection and radiotherapy and was alive until his last follow-up, 3.5

years after the diagnosis. However, a suspicious mass (6 mm) was detected after 27 months in the left frontal lobe (gyrus) but was not biopsied because of the location (too close to the speech zone) and risk of aphasia. The mass progressed to 13 mm after 12 months. He is still alive 15 months after the diagnosis of this irresectable mass.

A girl aged 8 years developed a symptomatic glioblastoma, 8 months after a normal MRI. She underwent surgery, radiotherapy and chemotherapy. There was a local recurrence 11 months after the initial diagnosis which was treated with radiotherapy. The patient developed liver failure after 4 months with a suspected diagnosis of haemophagocytic lymphohistiocytosis and died due to the complications of progressive liver failure. One patient accepted surveillance of the digestive tract but refused MRI screening of the brain. After 4 years of follow-up, he developed a glioblastoma at the age of 31 years and underwent surgery, radiotherapy and chemotherapy. He died from the complications of the tumour, 22 months after initial diagnosis.

Surveillance of the digestive tract

The protocol recommendations comprise annual gastroduodenoscopy and video capsule endoscopy (VCE) from the age of 10 years and annual colonoscopy from age 8 years. Twenty of the 22 patients underwent regular colonoscopies and 19 regular upper gastrointestinal (GI) endoscopy. Eight patients had at least one VCE.

Upper digestive tract

Duodenal adenoma with low-grade dysplasia were detected in five patients with maximal size of 20 mm which were endoscopically resected. Another two patients had hyperplastic duodenal polyps. One patient had multiple adenomatous polyps of maximal 20 mm with high-grade dysplasia and mucosectomy was planned for the patient. The median age of duodenal polyp diagnosis was 18.6 years (range: 10.1–28.1).

Two upper GI cancers were detected at the first screening. In one patient aged 23 years, a T3N1 gastric cancer was found. The patient underwent neo-adjuvant

chemotherapy and surgery and is still alive 3 years after diagnosis with no abnormalities detected on follow-up endoscopies. In the second patient, a T1N0 oesophageal cancer was diagnosed at the age of 18 years. She underwent neo-adjuvant chemotherapy and an oesophageal-cardia resection with gastric tube reconstruction but the patient died after 16 months due to the complications of glioblastoma. In another patient aged 10 years, gastric cancer of 4.2 cm located in the antrum was diagnosed during screening 16 months after the previous upper GI endoscopy. The patient was diagnosed with colon cancer with metachronous liver metastases 3 years earlier, before the diagnosis of CMMRD and start of the screening programme. The family refused treatment of the gastric cancer and the patient died <2 months after the diagnosis.

VCE detected polyps in two patients. Because the polyps were small, no additional diagnostic examinations were performed. During follow-up, one patient not under VCE surveillance developed a symptomatic SBC at age 26 (stage: pT3N2M1) located in the jejunum. The patient underwent surgery and chemotherapy and was alive at last follow-up, 4 years after the diagnosis.

Lower digestive tract

Multiple adenomas in the colon were found in 12 patients at the median age of 16.3 years (range: 9.0–29.6). The number of adenomas varied from 1 to 30 adenomas. The adenomas were equally distributed in the colon. In three patients, adenomas showed high-grade dysplasia. All polyps were endoscopically removed. The programme detected CRC in two patients. In one patient at the age of 19 years, a malignant polyp was endoscopically removed. She subsequently underwent a subtotal colectomy and no residual malignancy was detected (TNM: pT1N0). She died due the complications of a glioblastoma 10 months after the diagnosis of CRC. In another patient aged 28 years with a history of simultaneous rectal and colon malignancies 12 years ago, multiple adenomas were resected from the pouch, 11 months after the previous coloscopy followed by resection of the pouch. Pathological examination revealed an adenocarcinoma (TNM: pT2N2).

Tumours that develop at other sites

In our programme, there are no specific tools for the early detection of haematological disorders except checking medical history and blood investigation during the regular 6 monthly visits and optional abdominal ultrasound. One patient developed a myelodysplastic syndrome and died due to the syndrome at age 15 years. Two other patients were diagnosed with acute lymphoblastic leukaemia (ALL) at age 12 and 14 years and another patient with T-cell lymphoma (Ann Arbor stage IV) at the age of 8 years. T-cell lymphoma (mediastinal, testicular and medullar) was treated by conventional chemotherapy. After treatment, there was complete remission of testicular and mediastinal locations but persistent minimal residual disease detected by immunophenotyping. One of the patients with ALL (precursor B) underwent chemotherapy (according to AIEOP-BFM ALL 2017 protocol) plus allogenic haematopoietic stem cell transplantation and complete remission was achieved. All patients were alive and under treatment during the last follow-up screening.

DISCUSSION

In the current study, we present the outcome of surveillance of 22 patients with CMMRD. During a mean follow-up of 4 years, the programme detected eight malignant tumours including three brain tumours, three upper GI cancers and two CRCs. Most tumours could successfully be treated. In addition, adenomas were detected and subsequently removed involving colorectal adenomas in 12 patients and duodenal adenomas in 6 patients. Seven patients developed a symptomatic malignancy, comprising two brain tumours, one SBC and four haematological malignancies. At the end of the follow-up of 4 years, 16 out of 22 patients (73%) who participated in the surveillance programme were still alive.

The International Replication Repair Deficiency Consortium recently reported the results of surveillance in 53 patients with CMMRD who were prospectively followed in centres all over the world.[8] The study demonstrated that the 5-year overall survival significantly increased when cancers were detected asymptomatically (90% in asymptomatic vs 50% in symptomatic cancers).

In our surveillance programme, five patients were diagnosed with brain tumours including two patients diagnosed after presentation with symptoms. Two patients with a screen-detected tumour are still alive 2.5 and 3 years, respectively, after diagnosis. In the International Consortium cohort, a total of 20 brain tumours were diagnosed, including 5 patients with symptomatic tumours. The 5-year survival was twice as high (72% vs 33%) in patients with asymptomatic brain tumours compared with those with symptomatic tumours. In conclusion, both studies suggest that intensive surveillance with MRI scanning improves the prognosis and that not all tumours can be detected at an early stage and cured.

In the prospective cohort followed by the International Consortium, all GI cancers were detected in asymptomatic patients. The 5-year survival of the patients with asymptomatic tumours was 100%. The type of GI cancers was not specified in this study.[8] In the present European study, two patients were diagnosed with early stage gastric and oesophageal cancers on first screening. In a third patient, advanced gastric cancer was detected in a patient already diagnosed with liver metastases of CRC. In addition, benign duodenal lesions were detected and removed endoscopically in 27% of our patients. In the recent Consortium studies, no cancers of oesophagus or stomach were reported.[9, 10] We are not able to make conclusions on the value of VCE in the present study because in only 2 of 10 patients who underwent this procedure small polyps were detected and 1 patient developed a symptomatic SBC outside the programme. However, the role of VCE in CMMRD surveillance has recently been studied in 17 patients by Shimamura *et al*.[10] Polypoid lesions were detected in 63 % of VCEs (24/38) conducted on nine patients. Further investigation of three patients led to the detection of one adenocarcinoma in the jejunum in a patient aged 16 years. During the programme, two other patients were diagnosed with SBC detected with magnetic resonance enterography and upper GI endoscopy, respectively. The investigators concluded that although VCE was found to be effective to detect neoplasia of the small intestine, incomplete studies in 28%, false negative and positive results were found to be limiting factors of this screening modality.[10]

Colonoscopic surveillance in our cohort led to the detection and removal of many adenomas including adenomas with high-grade dysplasia. One early asymptomatic CRC as well as one advanced CRC were found. In a previous Consortium study by Aronson *et al*, the results of GI surveillance were reported for 24 patients.[9] Two CRC and two SBC were detected during surveillance. None of the patients undergoing surveillance died of GI malignancy. Levi *et al* reported the outcome of colonoscopy in 11 patients with CMMRD. Two patients were found to have CRC, three multiple (polyposis-like) polyps, four a few polyps and two no polyps, demonstrating the high yield of such a programme.[11] In conclusion, the benefits and effectiveness of colorectal surveillance are supported by our observations and previous studies.

Regarding haematological disorders, all of our cases were diagnosed after the presentation of symptoms. In the Consortium study, most (10 out of 12) haematological disorders were symptomatic.

An important question is, how the results of our and other recent studies can be used to improve the current surveillance protocols? [4–7]

For brain tumours, it is currently recommended to start the MRI scanning at 2 years of age.[5-7] Guerrini-Rousseau *et al* reported that among 49 patients with brain tumours known in the European C4CMMRD database, there was only one patient diagnosed with a brain tumour (medulloblastoma at age 1 year) before age 2.[12] In the present study, and also in the International Consortium study,[8] all tumours were detected beyond this age. In conclusion, all studies suggest that the current starting age limit is appropriate. Regarding the interval of MRI surveillance, in two of our patients, an astrocytoma and oligodendroglioma were detected within 6 months after the last MRI and both tumours could successfully be resected. Both patients are still alive. In two other patients, the brain tumours, both glioblastomas, were diagnosed at an interval longer than 6 months after 8 and 12 months, respectively. Both patients developed local recurrences within 12 months. In the prospective cohort of the International Consortium, 20 brain tumours were diagnosed of which 15 were asymptomatic and 5 were symptomatic. One of the five patients with symptomatic tumours had an surveillance interval of 1.5 years and in four of the five patients, the MRI schedule

was disrupted because of access or availability to surveillance modalities. It is unclear whether all asymptomatic tumours were detected within the 6 months interval. In conclusion, the few data available may suggest that a surveillance interval of 6 months should be recommended but in clinical practice, it may be challenging to perform MRI scans at such short intervals.

In our study, the four patients that developed gastric, oesophageal and SBC were between 10 and 26 years of age. In the VCE study by Shimamura *et al*,[10] three SBC were diagnosed at age 12, 15 and 20 years. The information on all known patients in the International Consortium study revealed that there were 10 patients with SBC of which 1 patient was diagnosed at age 9 and 2 patients with gastric cancer, of which 1 was diagnosed at age 9.[8] In our previous study, all 18 SBC were diagnosed >10 years.[5] In view of the rarity of upper GI and SBCs diagnosed before age 10, starting upper GI endoscopy and VCE from this age appears to be appropriate. However, if colonoscopy is performed under general anaesthesia (which is recommended in children), it may be considered to start performing upper GI endoscopy together with colonoscopy from the age of 8 years (see below) because there is no additional burden for the patients. There are insufficient data to evaluate the recommended interval of upper GI surveillance of 1 year, but experience suggests that this is appropriate.

Recommendations regarding the annual interval of colonoscopic screening and shorter intervals in case of multiple or advanced polyps seem also justified. However, there is discrepancy regarding the starting age for colonoscopic surveillance. In previous guidelines, colorectal surveillance as early as 3 and 6 years was recommended.[4-7] In our 2014 study, among 59 patients with CRC, there were no cases diagnosed below the age of 8 years.[5] Also in our current study and in the International Consortium study, there were no cases of CRC below age 8 years which suggest that starting surveillance at this age is appropriate.

In view of cancers that may develop outside the usual sites of tumours, whole body MRI (WBMRI) has been recommended by the International Consortium. In the recently published study, all brain tumours were detected by the brain MRI as well as the total body scan. In addition, one malignant tumour (type of tumour

not specified) was detected by WBMRI.[8] In our study, we did not observe the development of tumours outside the usual sites. More studies are needed to prove the effectiveness of total body MRI.

The present study has advantages and disadvantages. Advantages were the prospective collection of the data, the availability of detailed findings and the relatively long follow-up. A disadvantage might be the low number of patients participating in the programme primary due to the rarity of the syndrome. However, despite this low number, the patients developed a very high number of 15 tumours during follow-up.

CMMRD is one of the most lethal and devastating forms of hereditary cancer. Surveillance programmes may alleviate the tumour burden and improve the prognosis as demonstrated in the present and other studies. Future studies are needed to evaluate whether the adjustments of the surveillance protocol as suggested will lead to further increase of life expectancy.

ETHICS STATEMENTS

Ethics approval

The study was approved by the members of the C4CMMRD group at their meeting in Innsbruck, Austria, on 1 February 2014. The C4CMMRD database has been assessed by the local data protection officer at Gustave Roussy Institute to be in accordance with the reference methodology of (MR004) of the Commission Nationale Informatique et liberté (CNIL) and approved by the ethics committee 'CEEI Inserm' (IRB00003888). All living patients gave signed informed consent to use their data in the context of care, including international collaborations. For deceased patients, the ethics commission authorised the use of the data subject to a non-opposition which has been verified with their referring physician.

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CHAPTER 7

Summary and Discussion

SUMMARY AND DISCUSSION

This thesis aims to improve our understanding of the genetic and clinical aspects of inherited syndromes associated with adenomatous polyposis (APC-associated polyposis, MUTYH associated polyposis, and Constitutional Mismatch Repair Deficiency Syndrome) in order to optimize surveillance and management and to improve life expectancy of these patients.

GENETIC MODIFIERS OF COLONIC PHENOTYPE IN APC- ASSOCIATED POLYPOSIS

APC-associated polyposis is an autosomal dominant cancer predisposition syndrome caused by a germline pathogenic variant in the APC gene. Colonic manifestations of FAP include the development of hundreds to thousands of adenomatous polyps. Correlation between the mutation site in the APC gene and the colonic phenotype of FAP is well-established.[1] However, the phenotypic variability observed in patients with the same APC mutation suggests that beside genotype, other factors modify disease phenotype in APC mutation carriers. The role of modifier genes in disease severity of FAP has been studied and several modifiers have been suggested.[2–5] Yanaru-Fujisawa *et al.* reported the association of Phospholipase A2 Group IIa and fundic gland polyposis in patients with familial adenomatous polyposis.[5] In another study, Crobtree *et al.* suggested that polymorphisms in NAT1 and NAT2 variants may explain the severity of colonic FAP.[6]

In **chapter 2**, we investigated whether Single Nucleotide Polymorphisms (SNPs) that are associated with CRC in the general population might influence colonic phenotype in APC mutation carriers. We genotyped 16 CRC-associated SNPs identified by Genome wide association studies (GWAS) in a cohort of 419 APC mutation carriers and compared allele frequency of these SNPs in patients with less than 100 and more than 100 adenomas. In this study, we identified two CRC-associated SNPs, rs16892766 (8q23.3) and rs3802842 (11q23.1), which show an association with adenoma number in APC mutation carriers. Carriage of the C allele at 8q13.1 (rs16892766) showed a trend of association with ≥100 polyp group. A borderline association was observed in the codominant inheritance

model for rs3802842 and carriers of the risk allele of this SNP were also more frequent in the more severe phenotype group. We then tested the joint association of these two SNPs and both remained borderline significant using dominant mode of inheritance however the interaction of the two SNPs was not significant. Furthermore, we compared the total number of sporadic CRC risk alleles in both groups and the mean number of risk alleles was similar.

CD36 polymorphisms have recently been studied in patients with APC mutation and an association with disease onset was found in these patients.[7,8] In the first study by Holmes *et al*, three SNPs in CD36 (rs1049673, rs1761667 and rs1984112) were tested in 275 FAP patients.[7] In a follow-up study by Corner *et al* on CD36 SNPs in larger cohort of 395 FAP patients, a significant difference in the age of disease onset was seen in patients with APC mutation in the MCR region and homozygous wildtype allele in rs198411 SNP.[8]

Since our study, new CRC-associated SNPs have been identified but their association with the phenotype of FAP has not yet been investigated.[10,11]

In conclusion, it is apparent that SNPs can have a modifying effect on disease phenotype, affecting the age of onset and severity of polyposis. Identifying these modifiers could greatly enhance the personalized management of FAP patients making the search for new modifiers of great importance. In addition, large multicenter studies are necessary to investigate the impact of newly discovered SNPs on the medical treatment of FAP patients.

TUMOR SPECTRUM IN APC MUTATION CARRIERS

Colorectal cancer (CRC) has been the main cause of morbidity and mortality in patients with pathogenic variants in the APC gene, however, colorectal screening and timely preventive colectomy in these patients has led to a substantial reduction in mortality due to CRC.[12–14] Patients with FAP also have increased risk of extracolonic cancers including duodenal cancer, hepatoblastoma, brain, thyroid, and pancreatic cancers. The risk of these cancers varies widely in previous studies.[15–18] Therefore, the value of extra-intestinal screening programs was unknown and there was no consensus regarding the need for additional surveillance recommendations for these cancers. In **chapter 3,** we examined the

tumor spectrum in patients with APC-associated polyposis to understand whether life expectancy of these patients can be further improved by extending of the existing surveillance programs to other organs such as the thyroid, stomach, liver or pancreas.

In this study on 582 APC mutation carriers, 85 extracolonic malignancies were diagnosed in 74 patients. The most common extracolonic cancers found were duodenal cancer and skin tumors. The frequency of other FAP-associated cancers such as cancer of the thyroid, liver (hepatoblastoma), brain and stomach were low. Among the benign lesions, the most frequent were fundic gland polyps, duodenal and gastric adenomas and desmoid tumors. The most prevalent cause of death was cancer (59%), with 42% of the cancer deaths due to CRC and 21% of the cancer deaths due to duodenal cancer. Other causes of cancer deaths were lung cancer in three patients, pancreatic cancer and cancer of unknown primary in two patients and brain tumor, gastric cancer, non-Hodgkin lymphoma and hepatocellular carcinoma in one patient. One patient died because from thyroid cancer at the age of 78 years. The second and third most common causes of death were cardiovascular disease (12.5% of all deaths) and desmoid tumors (10.7% of all deaths), respectively.

A (recent) systematic review and meta-analysis on the prevalence of thyroid disease in FAP concluded that although benign thyroid disease is common, thyroid cancer is infrequent and only a small subset of patients may benefit from screening ultrasound.[19] This finding is supported by the results of our study where thyroid cancer was documented in 1.5% and only one patient died at age 78 years. More recently, a retrospective study on the morbidity and mortality was conducted among 107 patients with familial adenomatous polyposis (FAP) in Japan. Cancer and/or desmoid tumor was reported in 59 % of patients and CRC was the leading cause of death (46%) followed by desmoid tumor, small intestinal cancer, ovarian cancer, duodenal cancer, and sepsis. However, all patients with gastric or thyroid cancer were alive at the last follow-up.[20] Considering the high incidence of benign thyroid disease, low prevalence of thyroid cancer and rare mortality in FAP patients,[17,18] it seems that thyroid

screening will cause additional burden and anxiety but no survival benefit for these patients.

Upper GI endoscopy starting at the age of 20-25 years is currently recommended for management of duodenal polyps however there are no recommendations regarding screening for intestinal cancers distal to duodenum as high-level evidence is lacking.[21–23] In our cohort, we also did not detect any malignancies of small intestine distal to the duodenum however a recent study on 107 FAP patients in Japan, three small intestinal malignancies were detected and was the cause of death in 2 patients.[20]

Regarding pancreatic cancer in FAP, screening is also not recommended as there is not high-quality evidence and individualized screening maybe appropriate if family history is present.[22,23] Routine hepatoblastoma screening is currently debatable and guidelines suggest that abdominal ultrasound and measurement of α-fetoprotein every 3–6 months during the first 5-10 year of life may be considered especially if family history is present.[22,23] In our study, 4 cases of hepatoblastoma were documented however there no mortality related to this malignancy.

On the basis of our study and data in the literature, we concluded that extending these surveillance programs to extraintestinal cancers will not further improve life expectancy in APC mutation carriers.

BARRETT'S ESOPHAGUS IN APC AND MUTYH ASSOCIATED POLYPOSIS

Barrett's esophagus (BE), characterized by replacement of squamous epithelium by columnar epithelium in distal esophagus, is thought to be responsible for most cases of esophageal adenocarcinoma (EAC). Chronic inflammation due to gastro-esophageal reflux disease (GERD) is the main cause of the BE and EAC. Other than GERD, abdominal obesity and smoking are known risk factors of Barrett's esophagus and associated adenocarcinoma.[24–27]

Genetic factors also play a role in BE and EAC.[28–31] High genetic correlation and polygenic overlap was reported between BE and EAC.[28] In addition, a genome-wide association study has identified three significant susceptibility loci in a combined group of cases with esophageal adenocarcinoma and Barrett's

esophagus.[31] Dai *et al* also reported a susceptibility locus which modifies the association of gastroesophageal reflux with Barrett's esophagus.[30]

MUTYH-associated polyposis caused by germline biallelic MUTYH mutations was first described in 2002.[32] Colonic phenotype of patients with MUTYH-associated polyposis (MAP) mostly resembles AFAP. Various studies reported an increased risk of extraintestinal malignancies including ovarian, bladder, and skin cancers and a trend of increased risk of breast cancer.[33] Benign cutaneous tumors, lipomas, and benign endometrial and breast tumors have also been found in MAP patients.[33]

Gatalica *et al.* reported a high frequency (16%) of Barrett's esophagus (BE) in patients with adenomatous polyposis coli (APC)-associated polyposis (FAP) in a small group of 36 patients.[34] Therefore, to better understand disease phenotype, we examined the prevalence of BE and EAC in patients with MUTYH mutations and also a large cohort with germline APC mutations in **chapter 4.**

We studied the prevalence of BE in 72 patients with MAP and 407 patients with FAP and available upper GI endoscopy and/or pathology reports. We demonstrated that the prevalence of BE in MAP patients was 9.7% which is > 5 times higher than reported in the general population. However, in contrast with the previous study, no increased frequency of BE was found in the FAP patients. Another observation of this study was that in two MAP patients, there appeared to be an accelerated progression from low-grade dysplasia into high-grade dysplasia and EAC, respectively.

In conclusion, this study demonstrates that the prevalence of BE is much higher in patients with MAP compared to the general population. The impaired MUTYH protein function that plays a role in the repair of DNA damage caused by oxidative stress such as GERD may explain the high frequency of BE in MAP. In contrast, the prevalence of BE is not increased in FAP patients.

Based on the results of our study we recommend to pay attention to the presence of BE in patients with MAP when upper GI-endoscopy is performed. If the observed acceleration of high-grade dysplasia and EAC development is confirmed in more studies, intensive follow-up should be considered in patients with BE.

SURVEILLANCE PROTOCOL AND OUTCOME IN CONSTITUTIONAL MISMATCH REPAIR DEFICIENCY SYNDROME

Constitutional mismatch repair deficiency (CMMRD) is an autosomal recessive hereditary cancer syndrome caused by biallelic MMR mutations. Although CMMRD is a rare condition, many more patients are currently being diagnosed due to the recent advancements in high throughput sequencing. The clinical presentation of CMMRD is variable, ranging from benign polyps and multiple café au lait maculae to a wide spectrum of malignancies beginning in childhood including brain, hematological or gastrointestinal.

Durno *et al.* and Sjursen *et al*. reported on the outcome of surveillance of three CMMRD patients over a period of 10 and 26 years respectively.[35,36] In this follow-up period, many malignant tumors such as CRC were diagnosed and treated. In addition, multiple premalignant tumors including high-grade and low-grade adenomatous polyps were detected and removed.

Until recently, no formal guidelines were available for the management of CMMRD patients. Therefore, the newly established European Consortium “Care for CMMRD” (C4CMMRD) decided to develop diagnostic criteria for CMMRD as well as guidelines for management and surveillance.

The WHO has defined a set of criteria that should be met before implementation of screening programs.[37] These criteria can also be applied to surveillance of individuals with a hereditary cancer syndrome. In **chapter 5**, we reviewed the literature and collected clinical data on the tumor spectrum of nearly all CMMRD patients published in the literature, i.e., 146 CMMRD patients from 91 families.[38] The most common cancers in these patients were CRC, brain tumors, hematological malignancies and small bowel cancers, respectively. We also examined the age distribution of the above-mentioned cancers. Using all information on the tumor spectrum, natural course and the age distribution of the malignancies, we evaluated whether surveillance of the various cancers associated with CMMRD complied with the WHO-criteria. Based on our study and discussions among the members of the European Consortium “Care for C4CMMRD", a surveillance protocol was proposed.[38]

The European Consortium, C4CMMRD, also decided to establish a European Registry of CMMRD patients in order to collect clinical data prospectively and allow clinical studies. One of these studies was to evaluate the effectiveness of the C4CMMRD surveillance protocol.

In **chapter 6** we describe the results of surveillance in 22 patients over a period of four years. Despite this short follow-up, the program detected eight malignant tumors including three brain tumors, three upper gastrointestinal cancers and two colorectal cancers. Most of these tumors could successfully be treated. In addition, seven patients developed a symptomatic malignancy, including two brain tumors, one small bowel cancer and four hematological malignancies. At the end of follow-up, 16 out of 22 (73%) patients who participated in the surveillance program are still alive. We compared our results with previous studies of the International Replication Repair Deficiency Consortium.[39–41] In particular, we evaluated the stage of the detected cancers in relation to the surveillance interval and also the age distribution of diagnosis of the cancers and discussed how the surveillance program can be improved.

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APPENDICES

A

Samenvatting en Discussie

Publications

Acknowledgments

Curriculum Vitae

SAMENVATTING EN DISCUSSIE

Dit proefschrift heeft tot doel ons begrip van de genetische en klinische aspecten van erfelijke syndromen geassocieerd met adenomateuze polyposis (APC-geassocieerde polyposis, MUTYH-geassocieerde polyposis en Constitutional Mismatch Repair Deficiency Syndrome) te vergroten om de surveillance en behandeling te optimaliseren en de levensverwachting van deze patienten te verbeteren.

GENETISCHE MODIFICATIE VAN COLONMANIFESTATIES BIJ APC-GEASSOCIEERDE POLYPOSIS

APC-geassocieerde polyposis is een autosomaal dominant kankerpredispositie syndroom dat wordt veroorzaakt door een kiembaanpathogene variant in het APC-gen. Colonmanifestaties van FAP omvatten de ontwikkeling van honderden tot duizenden adenomateuze poliepen. De correlatie tussen de mutatieplaats in het APC-gen en het colonfenotype van FAP is goed ingeburgerd.[1] De fenotypische variabiliteit die wordt waargenomen bij patiënten met dezelfde APC-mutatie suggereert echter dat naast het genotype, andere factoren het fenotype van de ziekte bij APC-mutatiedragers wijzigen. De rol van modificerende genen bij de ernst van de ziekte van FAP is bestudeerd en er zijn verschillende modificerende factoren voorgesteld.[2-5] Yanaru-Fujisawa *et al.* rapporteerden de associatie van fosfolipase A2 Groep IIa en fundusklierpolyposis bij patiënten met familiaire adenomateuze polyposis.[5] In een ander onderzoek, suggereerde Crobtee *et al.* dat polymorfismen in NAT1- en NAT2-varianten de ernst van colon-FAP kunnen verklaren.[6]

In hoofdstuk 2 hebben we onderzocht of Single Nucleotide Polymorphisms (SNPs) die geassocieerd zijn met CRC in de algemene populatie, mogelijk het fenotype van de dikke darm in APC-mutatiedragers kunnen beïnvloeden. We hebben 16 CRC-geassocieerde SNP's gegenotypeerd die werden geïdentificeerd door Genome Wide Association Studies (GWAS) in een cohort van 419 APC-mutatiedragers en vergeleken de allelfrequentie van deze SNP's bij patiënten met minder dan 100 en meer dan 100 adenomen. In deze studie identificeerden we twee CRC-geassocieerde SNP's, rs16892766 (8q23.3) en rs3802842 (11q23.1),

die een verband vertonen met het aantal adenomen bij APC-mutatiedragers. Dragerschap van het C-allel op 8q13.1 (rs16892766) vertoonde een trend van associatie met de ≥100 poliepgroep. Een borderline-associatie werd waargenomen in het codominante overervingsmodel voor rs3802842 en dragers van het risico-allel van deze SNP kwamen ook vaker voor in de meer ernstige fenotypegroep. Vervolgens hebben we de gezamenlijke associatie van deze twee SNP's getest en beide bleven borderline-significant met behulp van de dominante overervingswijze, maar de interactie van de twee SNP's was niet significant. Verder vergeleken we het totale aantal sporadische CRC-risico-allelen in beide groepen en het gemiddelde aantal risico-allelen was vergelijkbaar.

CD36-polymorfismen zijn recentelijk bestudeerd bij patiënten met APC-mutatie en bij deze patiënten werd een verband gevonden met de leeftijd van ontstaan van de ziekte.[7,8] In de eerste studie van Holmes et al. werden drie SNP's in CD36 (rs1049673, rs1761667 en rs1984112) getest in 275 FAP-patiënten.[7] In een vervolgonderzoek door Corner et al naar CD36 SNP's in een groter cohort van 395 FAP-patiënten, werd een significant verschil gezien in de leeftijd waarop de ziekte begon bij patiënten met een APC-mutatie in het MCR-gebied en een homozygoot wildtype allel in rs198411 SNP.[8]

Sinds onze studie zijn er nieuwe CRC-geassocieerde SNP's geïdentificeerd, maar hun associatie met het fenotype van FAP is nog niet onderzocht.[10,11]

Concluderend is het duidelijk dat SNP's een modificerend effect kunnen hebben op het fenotype van de ziekte, wat de aanvangsleeftijd en de ernst van polyposis beïnvloedt. Het identificeren van deze modifiers zou de gepersonaliseerde behandeling van FAP-patiënten aanzienlijk kunnen verbeteren, waardoor het zoeken naar nieuwe modifiers van groot belang wordt. Daarnaast zijn grote multicenter studies nodig om de impact van nieuw ontdekte SNP's op de medische behandeling van FAP-patiënten te onderzoeken.

TUMORSPECTRUM IN APC-MUTATIEDRAGER

Colorectale kanker (CRC) is de belangrijkste oorzaak van morbiditeit en mortaliteit bij patiënten met pathogene varianten in het APC-gen, maar colorectale screening en tijdige preventieve colectomie bij deze patiënten

hebben geleid tot een substantiële vermindering van de mortaliteit als gevolg van CRC.[12-14] Patiënten met FAP hebben ook een verhoogd risico op buiten het colon gelegen kanker, waaronder kanker van de twaalfvingerige darm, hepatoblastoom, hersen-, schildklier- en alvleesklierkanker. Het risico op deze kankers varieert sterk in eerdere studies.[15-18] Daarom was de waarde van extra-intestinale screeningsprogramma's onbekend en bestond er geen consensus over de noodzaak van aanvullende surveillance-aanbevelingen voor deze kankers. In hoofdstuk 3 onderzochten we het tumorspectrum bij patiënten met APC-geassocieerde polyposis om te begrijpen of de levensverwachting van deze patiënten verder kan worden verbeterd door de bestaande surveillanceprogramma's uit te breiden naar andere organen zoals de schildklier, maag, lever of pancreas.

In deze studie met 582 APC-mutatiedragers werden 85 buiten het colon gelegen maligniteiten gediagnosticeerd bij 74 patiënten. De meest voorkomende tumoren die werden gevonden, waren kanker van de twaalfvingerige darm en huidtumoren. De frequentie van andere FAP-geassocieerde kankers zoals kanker van de schildklier, lever (hepatoblastoom), hersenen en maag was laag. Van de goedaardige laesies waren de meest frequente fundic gland poliepen, duodenum- en maagadenomen en desmoïdtumoren. De meest voorkomende doodsoorzaak was kanker (59%), met 42% van de sterfgevallen door kanker als gevolg van CRC en 21% van de sterfgevallen door kanker als gevolg van duodenum kanker. Andere doodsoorzaken van kanker waren longkanker bij drie patiënten, alvleesklierkanker en kanker van onbekende primaire vorm bij twee patiënten en hersentumor, maagkanker, non-Hodgkin-lymfoom en hepatocellulair carcinoom bij één patiënt. Eén patiënt stierf als gevolg van schildklierkanker op 78-jarige leeftijd. De tweede en derde meest voorkomende doodsoorzaken waren respectievelijk hart- en vaatziekten (12,5% van alle sterfgevallen) en desmoïdtumoren (10,7% van alle sterfgevallen).

In een (recente) systematische review en meta-analyse van de prevalentie van schildklieraandoeningen bij FAP werd geconcludeerd dat hoewel goedaardige schildklieraandoeningen vaak voorkomen, schildklierkanker zelden voorkomt en dat slechts een kleine subgroep van patiënten baat kan hebben bij screening met

echografie.[19] Deze bevinding wordt ondersteund door de resultaten van onze studie waarbij schildklierkanker werd gedocumenteerd bij 1,5% en slechts één patiënt stierf op 78-jarige leeftijd. Meer recent werd in Japan een retrospectief onderzoek naar de morbiditeit en mortaliteit uitgevoerd onder 107 patiënten met familiaire adenomateuze polyposis (FAP). Kanker en/of desmoïdtumor werd gemeld bij 59% van de patiënten en CRC was de belangrijkste doodsoorzaak (46%), gevolgd door desmoïdtumor, dunnedarmkanker, eierstokkanker, darmkanker en sepsis. Bij de laatste follow-up waren echter alle patiënten met maag- of schildklierkanker nog in leven.[20] Gezien de hoge incidentie van goedaardige schildklieraandoeningen, de lage prevalentie van schildklierkanker en de zeldzame mortaliteit bij FAP-patiënten, lijkt het erop dat schildklierscreening extra belasting en angst veroorzaakt maar geen overlevingsvoordeel voor deze patiënten.[17,18]

Endoscopie van het bovenste gedeelte van het maagdarmkanaal vanaf de leeftijd van 20-25 jaar wordt momenteel aanbevolen voor de behandeling van poliepen in de twaalfvingerige darm, maar er zijn geen aanbevelingen met betrekking tot screening op darmkanker distaal van de twaalfvingerige darm, aangezien bewijs op hoog niveau ontbreekt.[21-23] In ons cohort hebben we ook geen maligniteiten van de dunne darm ontdekt distaal van de twaalfvingerige darm, maar een recent onderzoek bij 107 FAP-patiënten in Japan, werden drie maligniteiten van de dunne darm ontdekt en de doodsoorzaak bij 2 patiënten.[20]

Met betrekking tot alvleesklierkanker bij FAP wordt screening ook niet aanbevolen, aangezien er geen bewijs van hoge kwaliteit is en ge-ïndividualiseerde screening misschien aangewezen is als er een familie-geschiedenis aanwezig is.[22,23]

Routinematige screening op hepatoblastoom is momenteel discutabel en richtlijnen suggereren dat abdominale echografie en meting van α-foetoproteïne elke 3-6 maanden gedurende de eerste 5-10 levensjaren kan worden overwogen, vooral als er een familiegeschiedenis aanwezig is.[22,23] In ons onderzoek werden 4 gevallen van hepatoblastoom gedocumenteerd, maar er was geen sterfte gerelateerd aan deze maligniteit.

Op basis van ons onderzoek en gegevens in de literatuur concludeerden we dat uitbreiding van deze surveillanceprogramma's naar extra-intestinale kankers de levensverwachting van APC-mutatiedragers niet verder zal verbeteren.

BARRETT'S SLOKDARM IN APC EN MUTYH GEASSOCIEERDE POLYPOSE

Barrett-slokdarm (BE), gekenmerkt door vervanging van plaveiselepitheel door cilindrisch epitheel in distale slokdarm, wordt verondersteld verantwoordelijk te zijn voor de meeste gevallen van adenocarcinoom van de slokdarm (EAC). Chronische ontsteking als gevolg van gastro-oesofageale refluxziekte (GERD) is de belangrijkste oorzaak van BE en EAC. Afgezien van GORZ zijn abdominale obesitas en roken bekende risicofactoren voor Barrett-slokdarm en geassocieerd adenocarcinoom.[24-27]

Genetische factoren spelen ook een rol bij BE en EAC.[28-31] Er werd een hoge genetische correlatie en polygene overlap gerapporteerd tussen BE en EAC.[28] Bovendien heeft een genoombrede associatiestudie drie significante vatbaarheidsloci geïdentificeerd in een gecombineerde groep gevallen met adenocarcinoom van de slokdarm en de slokdarm van Barrett.[31] Dai et al rapporteerden ook een gevoeligheidslocus die de associatie van gastro-oesofageale reflux met de slokdarm van Barrett beïnvloedt.[30]

MUTYH-geassocieerde polyposis veroorzaakt door biallelische MUTYH-mutaties in de kiembaan werd voor het eerst beschreven in 2002.[32] Colon fenotype van patiënten met MUTYH-geassocieerde polyposis (MAP) lijkt grotendeels op AFAP. Verschillende onderzoeken rapporteerden een verhoogd risico op extra-intestinale maligniteiten, waaronder eierstok-, blaas- en huidkanker, en een trend van een verhoogd risico op borstkanker.[33] Goedaardige huidtumoren, lipomen en goedaardige endometrium- en borsttumoren zijn ook gevonden bij MAP-patiënten.[33]

Gatalica et al. rapporteerden een hoge frequentie (16%) van Barrett's slokdarm (BE) bij patiënten met adenomateuze polyposis coli (APC)-geassocieerde polyposis (FAP) in een kleine groep van 36 patiënten.[34] Om het ziektefenotype beter te begrijpen, onderzochten we daarom de prevalentie van BE en EAC bij

patiënten met MUTYH-mutaties en ook een groot cohort met kiembaan-APC-mutaties in hoofdstuk 4.

We bestudeerden de prevalentie van BE bij 72 patiënten met MAP en 407 patiënten met FAP en beschikbare rapporten over gastro-intestinale endoscopie en/of pathologie. We hebben aangetoond dat de prevalentie van BE bij MAP-patiënten 9,7% was, wat > 5 keer hoger is dan gerapporteerd in de algemene bevolking. In tegenstelling tot de vorige studie werd er echter geen verhoogde frequentie van BE gevonden bij de FAP-patiënten. Een andere observatie van deze studie was dat er bij twee MAP-patiënten een versnelde progressie leek te zijn van respectievelijk lichte dysplasie naar hoogwaardige dysplasie en EAC.

Concluderend toont deze studie aan dat de prevalentie van BE veel hoger is bij patiënten met MAP in vergelijking met de algemene bevolking. De verminderde MUTYH-eiwitfunctie die een rol speelt bij het herstel van DNA-schade veroorzaakt door oxidatieve stress zoals GORZ kan de hoge frequentie van BE in MAP verklaren. Daarentegen is de prevalentie van BE niet verhoogd bij FAP-patiënten.

Op basis van de resultaten van onze studie raden we aan om aandacht te besteden aan de aanwezigheid van BE bij patiënten met MAP wanneer endoscopie van het bovenste deel van het maagdarmkanaal wordt uitgevoerd. Als de waargenomen versnelling van hooggradige dysplasie en EAC-ontwikkeling in meer studies wordt bevestigd, moet intensieve follow-up worden overwogen bij patiënten met BE.

SURVEILLANCEPROTOCOL EN UITKOMST BIJ HET CONSTITUTIONEEL MISMATCH-REPARATIEDEFICIËNTIESYNDROOM

Constitutionele mismatch-reparatiedeficiëntie (CMMRD) is een autosomaal recessief erfelijk kankersyndroom dat wordt veroorzaakt door biallelische MMR-mutaties. Hoewel CMMRD een zeldzame aandoening is, worden er momenteel veel meer patiënten gediagnosticeerd vanwege de recente vorderingen op het gebied van high-throughput sequencing. De klinische presentatie van CMMRD is variabel, variërend van goedaardige poliepen en meerdere café au lait maculae

tot een breed spectrum van maligniteiten die beginnen in de kindertijd, waaronder hersenen, hematologische of gastro-intestinale.

Durno et al. en Sjursen *et al.* rapporteerde over de uitkomst van surveillance van drie CMMRD-patiënten over een periode van respectievelijk 10 en 26 jaar.[35,36] In deze follow-up periode werden veel kwaadaardige tumoren zoals CRC gediagnosticeerd en behandeld. Bovendien werden meerdere premaligne tumoren, waaronder hooggradige en laaggradige adenomateuze poliepen, opgespoord en verwijderd.

Tot voor kort waren er geen formele richtlijnen beschikbaar voor de behandeling van CMMRD-patiënten. Daarom besloot het nieuw opgerichte Europese Consortium “Care for CMMRD” (C4CMMRD) diagnostische criteria voor CMMRD te ontwikkelen, evenals richtlijnen voor behandeling en surveillance.

De WHO heeft een reeks criteria gedefinieerd waaraan moet worden voldaan voordat screeningsprogramma's worden ingevoerd.[37] Deze criteria kunnen ook worden toegepast bij de surveillance van personen met een erfelijk kankersyndroom. In hoofdstuk 5 hebben we de literatuur beoordeeld en klinische gegevens verzameld over het tumorspectrum van bijna alle CMMRD-patiënten die in de literatuur zijn gepubliceerd, d.w.z. 146 CMMRD-patiënten uit 91 families.[38] De meest voorkomende kankers bij deze patiënten waren CRC, hersentumoren, hematologische maligniteiten en kankers van de dunne darm. We onderzochten ook de leeftijdsverdeling van de bovengenoemde kankers. Gebruikmakend van alle informatie over het tumorspectrum, het natuurlijk beloop en de leeftijdsverdeling van de maligniteiten, evalueerden we of surveillance van de verschillende kankers geassocieerd met CMMRD voldeed aan de WHO-criteria. Op basis van onze studie en discussies tussen de leden van het Europees Consortium “Care for C4CMMRD”, werd een surveillanceprotocol voorgesteld.[38]

Het Europese Consortium, C4CMMRD, besloot ook om een Europees register van CMMRD-patiënten op te richten om prospectieve klinische gegevens te verzamelen en klinische studies mogelijk te maken. Een van deze onderzoeken was het evalueren van de effectiviteit van het C4CMMRD-surveillanceprotocol.

In hoofdstuk 6 beschrijven we de resultaten van surveillance bij 22 patiënten over een periode van vier jaar. Ondanks deze korte follow-up ontdekte het programma acht kwaadaardige tumoren, waaronder drie hersentumoren, drie kankers van het bovenste deel van het maagdarmkanaal en twee darmkankers. De meeste van deze tumoren konden met succes worden behandeld. Bovendien ontwikkelden zeven patiënten een symptomatische maligniteit, waaronder twee hersentumoren, één dunnedarmkanker en vier hematologische maligniteiten. Aan het einde van de follow-up zijn 16 van de 22 (73%) patiënten die deelnamen aan het surveillanceprogramma nog in leven. We vergeleken onze resultaten met eerdere studies van het International Replication Repair Deficiency Consortium.[39-41] We evalueerden met name het stadium van de gedetecteerde kankers in relatie tot het surveillance-interval en ook de leeftijdsverdeling van de diagnose van de kankers en discussieren over hoe de surveillance programma kan worden verbeterd.

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CURRICULUM VITAE

Zeinab Ghorban Oghli is born on 18th February 1983 in Tehran, Iran. She completed Grade 11 at Tazkiyeh High school and the moved to South Africa and matriculated at Pretoria High School for Girls in 2000. She then started studying Medicine at the University of Witwatersrand (Johannesburg, South Africa) in 2001 and graduated in December 2006 with Bachelor of Medicine and Surgery (MBBCh). Thereafter, she moved back to Iran and attended 18 months of medical internship at Iran University of Medical Sciences (Tehran, Iran) and was then licensed to practice as a medical doctor by the Islamic Republic of Iran Medical Council.

Zeinab moved to the Netherlands in 2011 when she decided to peruse her education by applying for Master of biomedical science but she was advised to start her PhD. She then started her research in the field of hereditary cancers at Leiden University Medical School (LUMC) and Dutch Foundation for Hereditary Tumours (StOET) under supervision of Prof. Hans Vasen. During her PhD study, she presented the results of her research in international conferences and also the meetings of the European Consortium 'Care for CMMRD' (C4CMMRD). During her PhD study, Zeinab also helped to establish the Middle East Network on Hereditary Colorectal Cancer (https://www.hccn-me.com) with the goal to improve care for these high-risk groups in the Middle East and Eastern Mediterranean and North African Countries which also resulted in two publications.