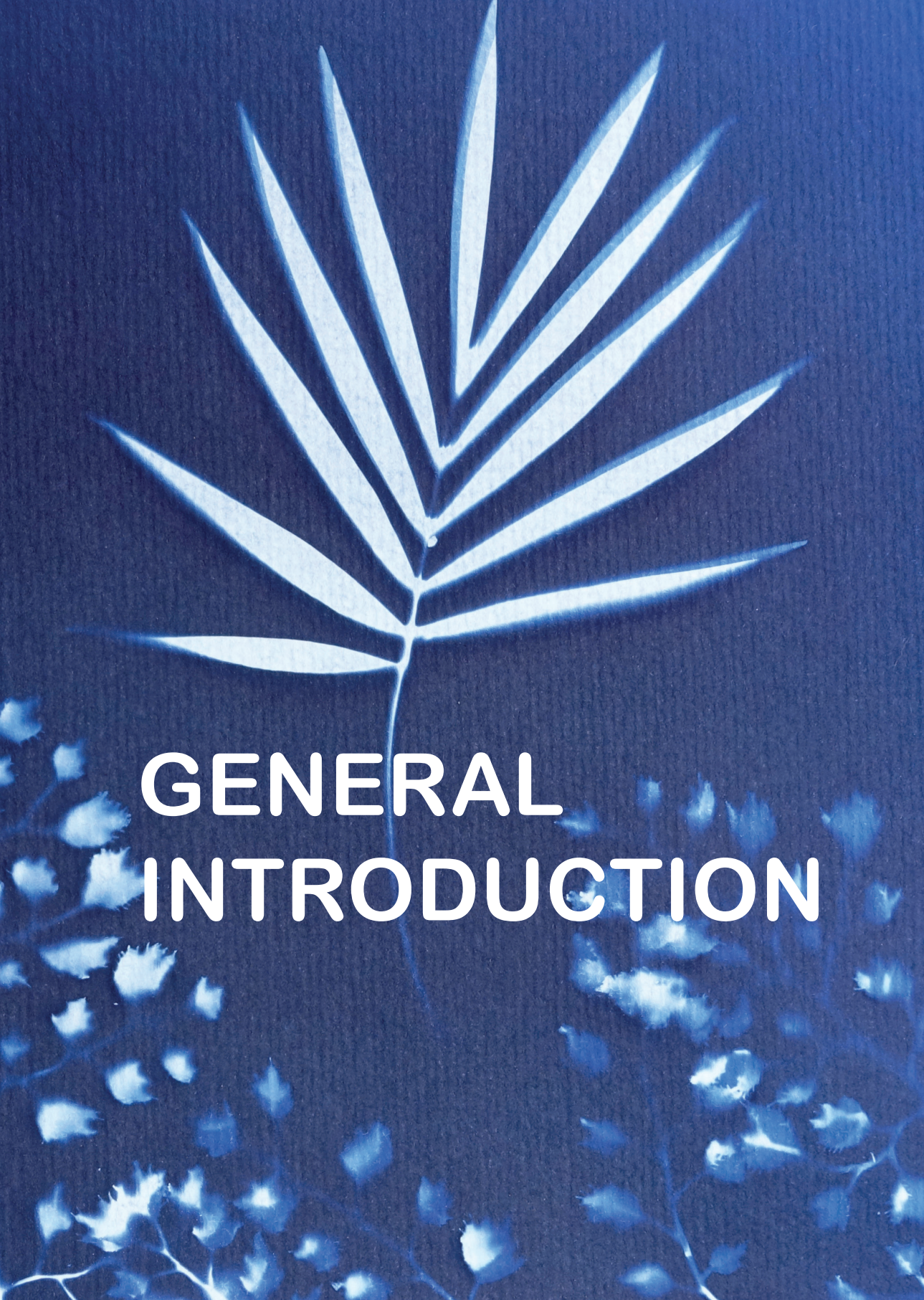
PMS2-associated Lynch syndrome : the odd one out

Broeke, S.W. ten


1



GENERAL INTRODUCTION

INTRODUCTION

Colorectal cancer is the third most frequently identified cancer worldwide and accounted for nearly 1.4 million new cases in 2012. This is expected to rise to 2.4 million new cases diagnosed each year by 2035.[1] The incidence of colorectal cancer in more developed countries is almost three times higher when compared to less developed countries suggesting that unhealthy lifestyle is a major contributor to colorectal cancer development.[1] However, in addition to environmental factors, hereditary factors play a role in the etiology in about 20-30% of colorectal cancer patients.[2] Approximately five percent of all colorectal cancer cases are associated with a highly penetrant inherited syndrome such as Lynch syndrome, familial adenomatous polyposis (FAP) or *MUTYH*-associated polyposis (MAP) (figure 1).[3] This thesis focusses on Lynch syndrome, in particular an underreported and under-investigated subtype, namely that associated with heterozygous pathogenic germline variants in the *PMS2* gene.

Lynch syndrome

Lynch syndrome (MIM 120435; formerly known as Hereditary Non-Polyposis Colorectal Cancer (HNPCC)) is the most common colorectal cancer predisposition syndrome, responsible for almost 3% of colorectal cancer and 2.5% of all endometrial cancer cases.[4,5] This syndrome is characterized by the development of colorectal and endometrial cancer with cumulative risks up to age 70 of 33-55% for colorectal and 10-45% for endometrial cancer.

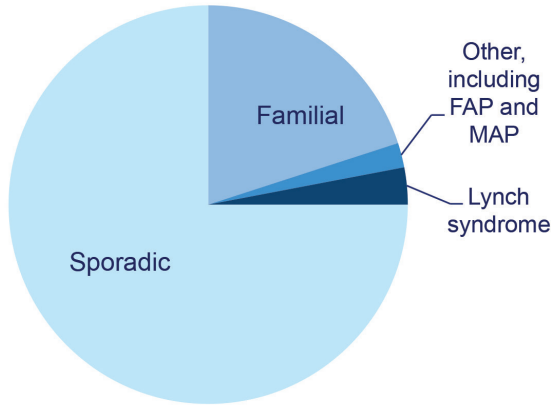


FIGURE 1 Pie-chart representing all diagnosed colorectal cancers.

Moreover, Lynch patients usually present with cancer at a relatively young age compared to the general population.[6] Other malignancies that occur significantly more frequently in Lynch patients than in the general population include ovarian, pancreatic, gastric, urinary tract, prostate and possibly breast cancer.[6-9] Lynch syndrome shows dominant inheritance and the underlying cause is a heterozygous pathogenic germline variant in one of the genes involved in DNA mismatch repair (MMR). These genes include *MLH1* (MIM 120436), *MSH2* (MIM 609309), *MSH6* (MIM 600678) and *PMS2* (MIM 600259). A recent addition to the list of genes causing Lynch syndrome is the epithelial cell adhesion molecule (*EPCAM*) gene, which, when deleted, inactivates *MSH2*.[10,11] Notably, biallelic pathogenic germline variants in the MMR genes cause a recessive form of childhood cancer that has been referred to as constitutional mismatch repair deficiency (CMMR-D) syndrome.[12,13]

In healthy individuals MMR proteins function as heterodimers in two main complexes consisting of MutS homologues MSH2 and either MSH6 or MSH3, and MutL homologues MLH1 binding to PMS2, PMS3, or MLH3. The MutS complex recognizes a mismatch and recruits the MutL complex which then initiates repair. These complexes act together in repairing mismatches and insertion-deletion loops.[14] [15,16] Tumors in Lynch patients arise or progress when the remaining wild type *MLH1*, *MSH2*, *MSH6* or *PMS2* allele is deactived because of a second hit or another truncating event, in line with Knudson's two hit hypothesis.[17] This leads to impaired MMR and subsequent accumulation of somatic variants in other (cancer) genes, which can eventually lead to uncontrolled cell growth and cancer. Hallmarks of these tumors as a result of faulty MMR are the shortening and lengthening of microsatellite regions, referred to as microsatellite instability (MSI), and the absence of MMR protein expression by immunohistochemistry (IHC). These changes allow patients with Lynch syndrome to be identified by the testing of tumors for these specific events.[3,18]

The exact percentage of individuals with an MMR germline variant in the general population is unknown. It has been estimated that the population incidence of Lynch syndrome could be as high as 1 in 370, based on the 2.8% incidence of Lynch syndrome among newly diagnosed colorectal cancer patients[4] and the 5% lifetime risk for colorectal cancer in the Western world. A recent report even estimated the prevalence to be up to 1 in 279.[19] Identifying individuals with Lynch syndrome is important, since clinical surveillance of this group can reduce colorectal cancer mortality by 70%.[20] Current surveillance protocols are identical for all MMR genes and include colonoscopies starting at age 20-25, every 1-2 years. For female MMR carriers biennial transvaginal ultrasounds with biopsies of the uterus can be considered, however there is no convincing evidence that this leads to improved survival.[21] Indeed, survival for

female Lynch patients with endometrial cancer is already very high. A recent report from the Prospective Lynch Syndrome Database (PLSD) estimated 10-year survival to be up to 93% (95% CI: 85%-97%). This database includes Lynch syndrome patients undergoing regular surveillance. Interestingly, *MLH1*, *MSH2* and *MSH6* carriers still had relatively high colorectal cancer risk (46%, 43% and 15% up to age 75, respectively) despite undergoing regular colonoscopies with polypectomies. It can thus be concluded that these polypectomies do not prevent all colorectal cancers for these MMR carriers, while interestingly, this does appear to be the case for *PMS2* carriers as Møller et al reported a 0% risk for this subset of Lynch patients.[22,23]

PMS2-associated Lynch syndrome

Since the first reports of the clinical involvement of *PMS2* in Lynch syndrome, it has been assumed that *PMS2* variants play only a minor role in Lynch syndrome.[24] This view has changed in recent years, as it has become clear that the role of *PMS2* variants has been underestimated. Even though the role of germline *PMS2* variants was described in 1994, clinical testing of the gene did not become available until 2009.[25-28] The reason for this is that the *PMS2* gene is notoriously difficult to analyze due to the existence of multiple pseudogenes. The gene is located on the short arm of chromosome 7 and spans 15 exons. Multiple regions with over 90% homology have been identified, all on chromosome 7. These pseudogene regions can interfere with sequencing of the *PMS2* gene. A variety of strategies, including the design of long-range amplicons[28] and RNA analysis[26], have helped to overcome this problem and have led to improved variant detection. Another explanation for the underestimation of PMS2-associated Lynch syndrome lies in the selection of families for genetic testing by family history or age of diagnosis (Bethesda and Amsterdam criteria)[29], whereas recent work has shown that *PMS2* variants are predominantly found in families that do not comply with these criteria.[30-32] While the identification of *PMS2* carriers in larger numbers seems to be improving, there are indications that many *PMS2* carriers remain to be identified. As the criteria defining Lynch syndrome are of limited use in the identification of these carriers, it may be necessary to examine population-based colorectal cancer cohorts.[32] Studies using IHC analysis in colorectal cancers from population-based cohorts have shown that isolated *PMS2* loss, indicative of a germline *PMS2* variants, is present in between 0.5-1.5% of unselected colorectal cancers.[31,32] The fraction of isolated PMS2 loss in MSI-high colorectal cancers varies between 1 and 8%.[33-35] One study of population-based colorectal cancers even found a higher percentage of abrogation of PMS2 than of MSH2 (12% versus 11%) in tumors with aberrant MMR staining.[32] More recent studies have also shown that in unselected (population-based) cohorts *PMS2*

and *MSH6* variants are much more prevalent (figure 2). Estimates of population carrier frequency based on statistical approaches are 1 in 714 and 1 in 758 for *PMS2* and *MSH6*, while the prevalence for *MLH1* and *MSH2* is 1 in 1946 and 1 in 2841, respectively.[19] This was confirmed in an unselected study involving the entire Icelandic population where they found an incidence of 1 in 226 for *PMS2* and *MSH6* variants combined.[36] Another indicator that the population frequency of *PMS2* carriers may possibly be even higher than that of *MLH1* and *MSH2* is the finding that biallelic *PMS2* variants comprise more than half of the homozygous or compound heterozygous variants in reported CMMR-D cases (31/57).[37] Although this might also be explained by the lower penetrance of *PMS2* variants, i.e. some biallelic *MLH1* or *MSH2* carriers may not be viable in utero.

The earlier under-representation of *PMS2* cases means that the clinical phenotype of carriers still requires further study. A small number of groups, including our own, studied cancer risk in this specific patient group.[31,38-40] In 2006, Hendriks et al published the first study on the clinical phenotype in *PMS2* carriers from our institution. Seven families with a previously unreported pathogenic *PMS2* variant were described in this study and it was shown that the mean age of colorectal cancer diagnosis for *PMS2* carriers was 52 years, 7-8 years higher than the mean age of diagnosis observed in families associated with *MLH1* and *MSH2* variants.[38] By including all available relatives in the penetrance analysis, irrespective of their mutation status (tested or not) an effort was made to

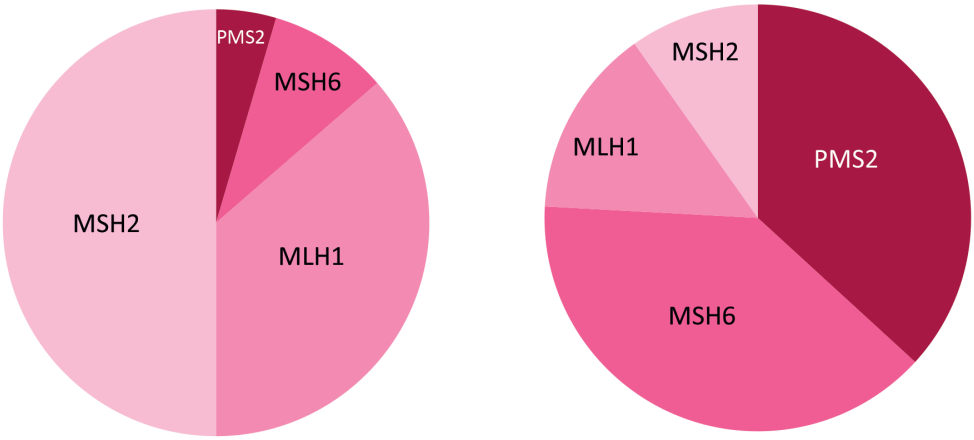


FIGURE 2 Estimated carrier frequencies in clinic- (left) vs. population- (right) based cohorts. Based on data reported by Win et al.[19]

reduce selection bias, but the inclusion of the index cases has likely still led to an overestimation of the cancer risk. Index cases which are almost always relatively young affected patients are unlikely to be representative for *PMS2* carriers in the general population. Therefore, when a low penetrance is to be expected, the inclusion of as many family members as possible on the one hand and the exclusion of index cases on the other, is of the utmost importance in order to limit selection bias. A large cohort of *PMS2* carriers (55 index patients and 55 relatives) was reported by Senter et al in 2008. This study reported a cumulative risk for colorectal cancer at age 70 of 20% (95% confidence interval: 11-34%) for male PMS2 carriers and 15% (95% confidence interval: 8-26%) for female *PMS2* carriers. The cumulative risk at age 70 for endometrial cancer was found to be 15%.[31] These are substantially lower risks than previously reported for *MLH1* and *MSH2* carriers. Reliable estimates on the risk for cancer outside the colon and endometrium will only become possible when larger studies are conducted. Risks estimated in larger studies are furthermore highly relevant for adequate surveillance policies. While Lynch families are currently advised to start surveillance between the ages of 20 and 25 and to continue with intervals of 1-2 years, less stringent surveillance guidelines may also be effective for *PMS2* carriers. The studies described in this thesis provide the basis for such PMS2-specific guidelines.

An essential next step to increase our understanding of the relatively low penetrance of *PMS2* variants is to study the role of this gene in carcinogenesis. Specific hallmarks of the molecular and immunological profile are to be expected and knowing these is relevant, since they can aid in a better identification of cases and might influence treatment and survival outcomes. Indeed, a lower somatic variant load in *PMS2* carriers compared to *MLH1* or *MSH2* carriers can be expected, since the MLH1/MLH3 heterodimer can partially compensate for the loss of the MLH1/PMS2 heterodimer. The high somatic variant load in MMR deficient tumors is thought to be the reason for an increased immune reaction surrounding these cancers, which in turn may explain improved survival in Lynch syndrome patients.[41] If there are indeed fewer somatic variants in PMS2 deficient tumors then this might result in a different involvement of the immune response which may consequently influence survival outcome. Moreover, a future possibility is that prophylactic vaccination of Lynch syndrome might elicit an early T-cell immune response, leading to tumor eradication or impeded tumor progression beyond the early stages.[42-44] Understanding of the role of specific T-cells in Lynch syndrome (including *PMS2*) could thus prove essential for the development of novel immunotherapeutic approaches. Lastly, the mechanism of the second hit in PMS2-asscoaiated cancers is often unknown; deletions, chromosomal loss and

methylation have all been suggested as possibilities.[45] More knowledge on this may also increase possibilities of cancer prevention.

Another clinical challenge is the fact that previous reports on *PMS2* have clearly shown a wide phenotypic variation within and between families. It therefore seems highly plausible that external and/or internal modifiers play a significant role in PMS2-related cancer development. Genome-wide association studies (GWAS) have uncovered numerous robust associations between common variants (single nucleotide polymorphisms, SNPs) and colorectal cancer risk. Researchers from our institution have demonstrated in 2009 that two SNPs, located on 8q23.3 (rs16892766) and 11q23.1 (rs3802842), are associated with an increased colorectal cancer risk in Dutch Lynch syndrome patients. This study revealed that patients homozygous for the minor allele of SNP rs16892766 showed an elevated risk of colorectal cancer in a dose-dependent manner, with a 2.16-fold increased risk of developing colorectal cancer. The CC variant genotype of SNP rs3802842 was also associated with an increased risk of colorectal cancer, but in female carriers only (HR=3.08). In a combined analysis of the two SNPs, risk was significantly associated with the number of risk alleles and the effect was shown to be stronger in female carriers than in male carriers.[46] This effect in Lynch syndrome patients was subsequently confirmed by an independent study in Polish and Australian Lynch syndrome patients.[47] Only one study included *PMS2* carriers, but only a limited number (n=40).[48] They found an increased risk for carriers of the G-allele of rs10795668 (10p14) and rs9929218 (16q22.1). Notably, this was the reversed effect as observed in the GWAS. Larger studies are needed to determine the role of SNPs in PMS2-associated Lynch syndrome.

Other well-known cancer risk factors, such as obesity and smoking might also contribute to the colorectal cancer risk and the development of adenomas.[49] It has been proposed that smoking is involved in epigenetic modification of MMR genes, and can thus serve as a second hit.[50] Indeed, smoking has been found to lead to an increased risk of carcinomas that show microsatellite instability and in a recent prospective study (GEOLynch cohort, consisting of *MLH1*, *MSH2* and *MSH6* carriers), smoking was shown to have a stronger effect on adenoma development in Lynch syndrome subjects than in sporadic cases (HR 7.1 for current smoking and 2.7 for former smokers).[51] A high BMI also leads to a higher tumor development in male Lynch subjects (HR 8.7).[52] Since *PMS2* has a reduced penetrance compared with other MMR genes, and also a still unexplained high clinical variance within families, lifestyle factors might be of even greater importance in this specific group. Since the previously mentioned studies did not include *PMS2* carriers further research on this hypothesis is needed.

Aim of this thesis

Although traditionally *PMS2* carriers were thought to account for only a minority of Lynch syndrome families, recent studies suggest significantly higher carrier frequencies of *PMS2* variants in the population when compared to *MLH1* and *MSH2*. We expect a rise in the identification of PMS2 families with the recent implementation of universal screening for MMR deficiency in all colorectal cancers below age 70. Unfortunately, the phenotype of PMS2-associated Lynch syndrome is poorly defined. This thesis describes epidemiological and molecular studies of PMS2 families and tumors in an attempt to further delineate the PMS2-associated phenotype thus providing enough evidence for gene-specific clinical guidelines.

1

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al: Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136:E359-86, 2015
2. Lichtenstein P, Holm NV, Verkasalo PK, et al: Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 343:78-85, 2000
3. Vasen HF, Tomlinson I, Castells A: Clinical management of hereditary colorectal cancer syndromes. Nat Rev Gastroenterol Hepatol 12:88-97, 2015
4. Hampel H, Frankel WL, Martin E, et al: Feasibility of screening for Lynch syndrome among patients with colorectal cancer. J.Clin.Oncol. 26:5783-5788, 2008
5. Hampel H, Frankel W, Panescu J, et al: Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. Cancer Res. 66:7810-7817, 2006
6. Barrow E, Hill J, Evans DG: Cancer risk in Lynch Syndrome. Fam.Cancer 12:229-240, 2013
7. Barrow E, Robinson L, Alduaij W, et al: Cumulative lifetime incidence of extracolonic cancers in Lynch syndrome: a report of 121 families with proven mutations. Clin. Genet. 75:141-149, 2009
8. Win AK, Lindor NM, Young JP, et al: Risks of primary extracolonic cancers following colorectal cancer in lynch syndrome. J.Natl.Cancer Inst. 104:1363-1372, 2012
9. Win AK, Young JP, Lindor NM, et al: Colorectal and other cancer risks for carriers and noncarriers from families with a DNA mismatch repair gene mutation: a prospective cohort study. J Clin Oncol 30:958-64, 2012
10. Ligtenberg MJ, Kuiper RP, Chan TL, et al: Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. Nat Genet 41:112-7, 2009
11. Kovacs ME, Papp J, Szentirmay Z, et al: Deletions removing the last exon of TACSTD1 constitute a distinct class of mutations predisposing to Lynch syndrome. Hum Mutat 30:197-203, 2009
12. Wimmer K, Etzler J: Constitutional mismatch repair-deficiency syndrome: have we so far seen only the tip of an iceberg? Hum.Genet. 124:105-122, 2008
13. Wimmer K, Kratz CP, Vasen HF, et al: Diagnostic criteria for constitutional mismatch repair deficiency syndrome: suggestions of the European consortium 'Care for CMMRD' (C4CMMRD). J.Med.Genet. 51:355-365, 2014

14. Peltomaki P: Role of DNA mismatch repair defects in the pathogenesis of human cancer. J.Clin.Oncol. 21:1174-1179, 2003
15. Jiricny J: The multifaceted mismatch-repair system. Nat Rev Mol Cell Biol 16. Peltomaki P: Update on Lynch syndrome genomics. Fam Cancer, 2016
17. Knudson AG, Jr.: Mutation and cancer: statistical study of retinoblastoma. Proc. Natl.Acad.Sci.U.S.A 68:820-823, 1971
18. Umar A, Boland CR, Terdiman JP, et al: Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J.Natl.Cancer Inst. 96:261-268, 2004
19. Win AK, Jenkins MA, Dowty JG, et al: Prevalence and Penetrance of Major Genes and Polygenes for Colorectal Cancer. Cancer Epidemiol Biomarkers Prev, 2016
20. de Jong AE, Hendriks YM, Kleibeuker JH, et al: Decrease in mortality in Lynch syndrome families because of surveillance. Gastroenterology 130:665-71, 2006
21. Renkonen-Sinisalo L, Butzow R, Leminen A, et al: Surveillance for endometrial cancer in hereditary nonpolyposis colorectal cancer syndrome. Int J Cancer 120:821-4, 2007
22. Moller P, Seppala T, Bernstein I, et al: Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database. Gut, 2015
23. Moller P, Seppala TT, Bernstein I, et al: Cancer risk and survival in path_MMR carriers by gene and gender up to 75 years of age: a report from the Prospective Lynch Syndrome Database. Gut, 2017
24. Peltomaki P: Role of DNA mismatch repair defects in the pathogenesis of human cancer. J Clin Oncol 21:1174-9, 2003
25. Nicolaides NC, Papadopoulos N, Liu B, et al: Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 371:75-80, 1994
26. van der Klift HM, Tops CM, Bik EC, et al: Quantification of sequence exchange events between PMS2 and PMS2CL provides a basis for improved mutation scanning of Lynch syndrome patients. Hum.Mutat. 31:578-587, 2010
27. van der Klift HM, Mensenkamp AR, Drost M, et al: Comprehensive Mutation Analysis of PMS2 in a Large Cohort of Probands Suspected of Lynch Syndrome or Constitutional Mismatch Repair Deficiency (CMMRD) Syndrome. Hum Mutat, 2016
28. Clendenning M, Hampel H, LaJeunesse J, et al: Long-range PCR facilitates the identification of PMS2-specific mutations. Hum.Mutat. 27:490-495, 2006
29. Vasen HF, Moslein G, Alonso A, et al: Recommendations to improve identification of hereditary and familial colorectal cancer in Europe. Fam Cancer 9:109-15, 2010

30. Clendenning M, Senter L, Hampel H, et al: A frame-shift mutation of PMS2 is a widespread cause of Lynch syndrome. J Med Genet 45:340-5, 2008
31. Senter L, Clendenning M, Sotamaa K, et al: The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. Gastroenterology 135:419-428, 2008
32. Truninger K, Menigatti M, Luz J, et al: Immunohistochemical analysis reveals high frequency of PMS2 defects in colorectal cancer. Gastroenterology 128:1160-1171, 2005
33. Hampel H, Frankel WL, Martin E, et al: Feasibility of screening for Lynch syndrome among patients with colorectal cancer. J Clin Oncol 26:5783-8, 2008
34. Baudhuin LM, Burgart LJ, Leontovich O, et al: Use of microsatellite instability and immunohistochemistry testing for the identification of individuals at risk for Lynch syndrome. Fam Cancer 4:255-65, 2005
35. Halvarsson B, Lindblom A, Rambech E, et al: The added value of PMS2 immunostaining in the diagnosis of hereditary nonpolyposis colorectal cancer. Fam.Cancer 5:353-358, 2006
36. Haraldsdottir S, Rafnar T, Frankel WL, et al: Comprehensive population-wide analysis of Lynch syndrome in Iceland reveals founder mutations in MSH6 and PMS2. Nat Commun 8:14755, 2017
37. Herkert JC, Niessen RC, Olderode-Berends MJ, et al: Paediatric intestinal cancer and polyposis due to bi-allelic PMS2 mutations: case series, review and follow-up guidelines. Eur.J.Cancer 47:965-982, 2011
38. Hendriks YM, Jagmohan-Changur S, van der Klift HM, et al: Heterozygous mutations in PMS2 cause hereditary nonpolyposis colorectal carcinoma (Lynch syndrome). Gastroenterology 130:312-322, 2006
39. Nicolaides NC, Papadopoulos N, Liu B, et al: Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 371:75-80, 1994
40. Niessen RC, Kleibeuker JH, Westers H, et al: PMS2 involvement in patients suspected of Lynch syndrome. Genes Chromosomes Cancer 48:322-9, 2009
41. de Miranda NF, Hes FJ, Van WT, et al: Role of the microenvironment in the tumourigenesis of microsatellite unstable and MUTYH-associated polyposis colorectal cancers. Mutagenesis 27:247-253, 2012
42. de Miranda NF, Goudkade D, Jordanova ES, et al: Infiltration of Lynch colorectal cancers by activated immune cells associates with early staging of the primary tumor and absence of lymph node metastases. Clin Cancer Res 18:1237-45, 2012
43. von Knebel Doeberitz M, Kloor M: Towards a vaccine to prevent cancer in Lynch syndrome patients. Fam Cancer 12:307-12, 2013

44. Kloor M, von Knebel Doeberitz M: The Immune Biology of Microsatellite-Unstable Cancer. Trends Cancer 2:121-133, 2016
45. Tomlinson IP, Roylance R, Houlston RS: Two hits revisited again. J Med Genet 38:81-5, 2001
46. Wijnen JT, Brohet RM, van ER, et al: Chromosome 8q23.3 and 11q23.1 variants modify colorectal cancer risk in Lynch syndrome. Gastroenterology 136:131-137, 2009
47. Talseth-Palmer BA, Wijnen JT, Brenne IS, et al: Combined analysis of three Lynch syndrome cohorts confirms the modifying effects of 8q23.3 and 11q23.1 in MLH1 mutation carriers. Int.J.Cancer 132:1556-1564, 2013
48. Win AK, Hopper JL, Buchanan DD, et al: Are the common genetic variants associated with colorectal cancer risk for DNA mismatch repair gene mutation carriers? Eur J Cancer 49:1578-1587, 2013
49. Liang PS, Chen TY, Giovannucci E: Cigarette smoking and colorectal cancer incidence and mortality: systematic review and meta-analysis. Int J Cancer 124:2406-15, 2009
50. Limsui D, Vierkant RA, Tillmans LS, et al: Cigarette smoking and colorectal cancer risk by molecularly defined subtypes. J Natl Cancer Inst 102:1012-22, 2010
51. Winkels RM, Botma A, Van Duijnhoven FJ, et al: Smoking increases the risk for colorectal adenomas in patients with Lynch syndrome. Gastroenterology 142:241-247, 2012
52. Botma A, Nagengast FM, Braem MG, et al: Body mass index increases risk of colorectal adenomas in men with Lynch syndrome: the GEOLynch cohort study. J.Clin.Oncol. 28:4346-4353, 2010