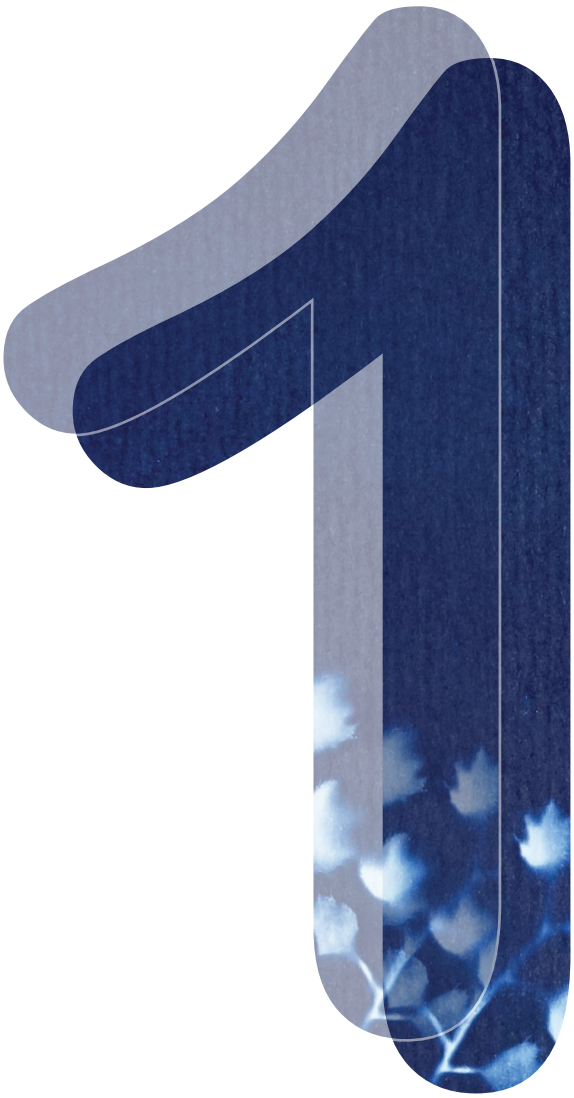
Germline variants in the mismatch repair genes: Detection and phenotype

Suerink, M.



Introduction

With 9.6 million estimated deaths in 2018, cancer is the second leading cause of death worldwide[1] and, in the Netherlands, the lifetime risk of developing at least one malignancy is about 1 in 3.[2,3] The most common types of cancer worldwide are lung cancer, breast cancer and colorectal cancer.[1] In some families, clustering of specific cancer subtypes suggests there are factors that increase cancer risk to a level well above population risk. Long before the underlying genes were discovered, it was suggested that a genetic predisposition to the development of cancer may explain the phenotype in at least a proportion of these families.[4-6] One of the most famous examples is Family G, a family that was described for the first time in 1913 by Aldred Scott Warthin, with a clustering of uterine and stomach cancers.[6] This large family intrigued medical professionals and has been described multiple times in the course of history. One of these professionals was Henry T. Lynch, who studied family G in detail and published several families with a similar history.[6] Over the past few decades the genetic basis for many of these syndromes, including the genetic cause in family G, has been unravelled; they are caused by germline pathogenic variants in genes that are important in the maintenance of genomic stability.[6-8]

We now know that the cancer predisposition syndrome responsible for the high cancer risk in family G is Lynch syndrome; an autosomal, dominantly inherited condition caused either by a germline pathogenic variant in one of four mismatch repair (MMR) genes (*MLH1, MSH2, MSH6* or *PMS2*)[9] or, more rarely, by a germline deletion of the ‘3 region of the *EPCAM* gene which silences the *MSH2* gene by hypermethylation.[10] In the case of family G, a germline variant in the *MSH2* gene was identified.[11] The MMR system plays a vital role in replication error correction in order to prevent mutations from accumulating. Replication error correction is carried out by MutS and MutL complexes that respectively recognize mismatches and activate downstream activities to initiate repair (Figure 1). MutS exists in two forms: as MSH2 coupled either with MSH6 to form MutSα or with MSH3 to form MutSβ. MutL exists as MutLα (MLH1•PMS2), MutLβ (MLH1•PMS1) and MutLγ (MLH1•MLH3).[12] While variants in *MLH1, MSH2, MSH6* and *PMS2* have been recognized to cause the dominantly inherited Lynch syndrome, variants in *MLH3* and *MSH3* have only been described in recessively inherited cancer syndromes.[13,14] The role of *PMS1* variants as a cause of cancer predisposition seems limited.[15] Homozygous and compound heterozygous pathogenic variants in *MSH2, MSH6, MLH1* and *PMS2* have also been described and result in a rare cancer predisposition syndrome called constitutional mismatch repair deficiency (CMMRD).[16,17]

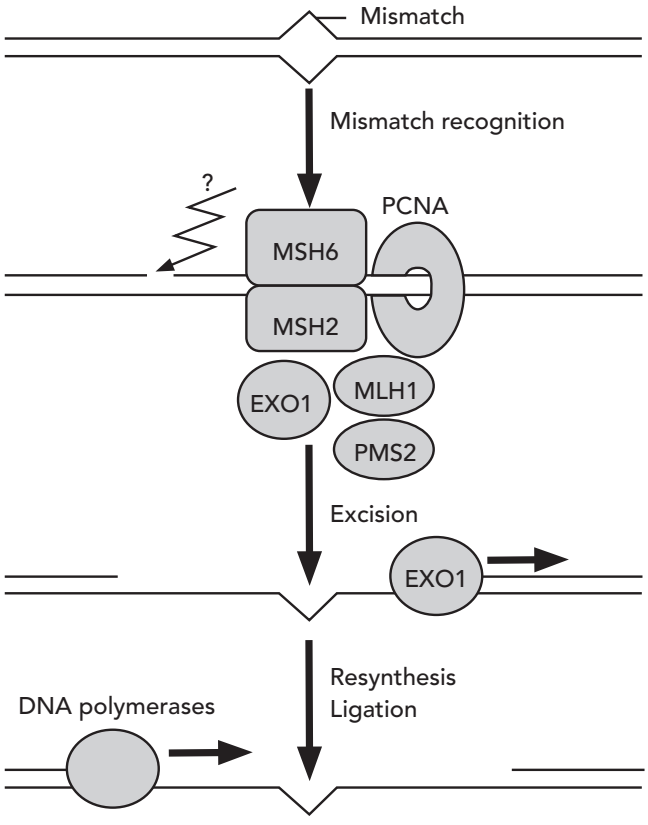


Figure 1 Mismatch recognition and repair by the mismatch repair genes. Reprinted with permission of Springer Nature.[18]

Lynch syndrome

Patients with Lynch syndrome mainly have an increased risk of developing colorectal and endometrial cancer during adulthood, but increased risks of developing cancer of the ovaries, small bowel, stomach, breast, hepatobiliary tract, prostate and urinary tract have also been reported.[19,20]

Previously, clinical criteria (such as the Amsterdam criteria and the (revised) Bethesda guidelines)[21,22] were used to preselect patients before genetic testing was performed. These criteria aimed at preselecting families with a higher a priori chance of a genetic predisposition by incorporating factors such as age at cancer diagnosis, type of cancer and positive family history.[21,23] Over time it became clear that many families do not

meet these criteria despite presence of a pathogenic variant.[24] Therefore, universal screening (sometimes also referred to as reflex testing) for MMR deficiency in colorectal cancer and endometrial cancer is becoming general practice in many countries in order to identify more variant carriers that could benefit from surveillance.[24-26] In the Netherlands all colorectal and endometrial cancers detected before the age of 70 are screened by immunohistochemical staining of the mismatch proteins and/or microsatellite instability analysis for the presence of MMR deficiency. If MLH1 deficiency is detected, *MLH1*-promotor hypermethylation analysis is first performed to rule out this epigenetic event as the somatic, sporadic cause. The presence of *MLH1*-promotor hypermethylation makes an hereditary cause of MMR deficiency highly unlikely, although germline cases have been described.[27-31] In absence of *MLH1*-promotor hypermethylation or when lack of MSH2, MSH6 or PMS2 protein expression is observed in the tumour, patients are referred to a clinical geneticist to further discuss genetic testing. Subsequent genetic testing will then have to determine whether the MMR deficiency is caused by a germline variant or by two somatic hits.

Although this approach facilitates detection of Lynch syndrome in families that do not meet clinical criteria such as the revised Bethesda criteria, it is likely that still many carriers remain unidentified. Recent estimates of carrier frequencies in the general population for pathogenic variants in the MMR genes are 1 in 1,946 for *MLH1*, 1 in 2,841 for *MSH2*, 1 in 758 for *MSH6* and 1 in 714 for *PMS2*, adding up to a total carrier frequency of 1 in 279.[32] This would mean that in a population of 17 million, such as the Netherlands, there should be almost 61.000 carriers. Identification of pathogenic variant carriers is crucial, since colonoscopic surveillance has been proven to be an effective, risk-reducing measure.[33] Of note, estimations of carrier frequencies are largely based on Western populations, in populations with large subpopulations of non-Western immigrants the carrier frequencies may differ.

Currently surveillance is offered in the same manner for all four genes with colonoscopic surveillance starting from age 20-25 years with an interval of 1-2 years,[23] but a plea for gene-specific guidelines is ongoing and will likely be implemented in the near future.[34-36] This was triggered by recent insights that the height of colorectal cancer risk varies depending on the mutated gene. Risks are highest for carriers of a pathogenic *MLH1* or *MSH2* variant with estimations of the colorectal cancer risk up to age 70 varying between 52% and 97%,[19] while these risks estimates are lower for *MSH6* (22-69%)[19] and lowest for *PMS2* (11-20%).[36-38] Prospective data further illustrate the difference in penetrance between the MMR genes: the risk of developing colorectal

cancer whilst being under surveillance, is still substantial (up to 57%) for *MLH1* and *MSH2* pathogenic variant carriers, while it is much lower (20%) for *MSH6* and seems to be very low (0-10.4%) for *PMS2*.[35,39]

The challenge with establishing correct cancer risks for any cancer predisposition syndrome, and Lynch syndrome is no exception, is that retrospective analyses are complicated by the fact that available patient cohorts have been heavily selected on family history and analyses require statistical methods to correct for this ascertainment bias.[40] Nonetheless, statistical methods come with limitations as well. This is nicely illustrated by a study in hereditary breast cancer, showing that much of the variation seen in breast cancer risk estimates can be explained by the use of different bias correction methods.[41] Large initiatives, such as the Prospective Lynch Syndrome Database (PLSD), have therefore been developed to gather prospective data on Lynch syndrome families.[39] Then again, these risk estimations are tricky to use in guideline development; they underestimate true colorectal cancer risk since study participants are undergoing surveillance and are therefore less likely to develop cancer. Further confirmation of previously reported (retrospective) risk estimates is therefore needed.

Gene specific risk stratification is one step in the right direction towards tailored surveillance guidelines, but even then room for improvement remains: large differences in penetrance have been observed between families and individuals with variants in the same gene. Statistical modelling indicates that there is large heterogeneity in cancer risk between *MLH1* and *MSH2* variant carriers with a large proportion (around a quarter) of carriers with a relatively low (0-10%) risk of developing colorectal cancer before the age of 70 and a smaller proportion (10-20%) at extremely high risk (90-100%) (Figure 2).[42] Many mechanisms have been suggested to explain these differences, including lifestyle factors,[43-45] risk modifying SNPs[42,46-48] and genotype-phenotype correlations[42,49-51], but none of these factors have been implemented in clinical practice yet. Further risk stratification would be desirable to reduce the burden of frequent colonoscopies for those with a low risk, while those with a higher risk are adequately targeted. Although there are no similar studies yet to provide evidence for a similar risk distribution in *PMS2* and *MSH6* families, clinical observations suggest similar risk distributions within these families, possibly with an even greater proportion of family members that fall in the lower-risk categories.

CMMRD

In CMMRD, the cancer spectrum is much broader and penetrance is much higher than in Lynch syndrome; cancer penetrance is virtually complete and patients often already present with cancer at very young ages (childhood or adolescence).[17] Apart from a high risk of Lynch syndrome associated cancers at a young age, other cancers risks that are strongly increased in these patients include those for tumours of the central nervous system and haematological malignancies.[17] A non-malignant clinical sign of CMMRD is the presence of café-au-lait macules (CALMs), which is why children with CMMRD are sometimes first suspected of neurofibromatosis type 1 before receiving the correct diagnosis.[52] Diagnostic criteria exist to identify CMMRD in those patients who have already developed cancer [17] and guidelines for surveillance of patients with CMMRD have been published.[53-55] Although more research is needed to definitively prove the efficacy of these surveillance guidelines, preliminary reports in a small series of patients show promising results.[56] Furthermore, the use of aspirin and neo-

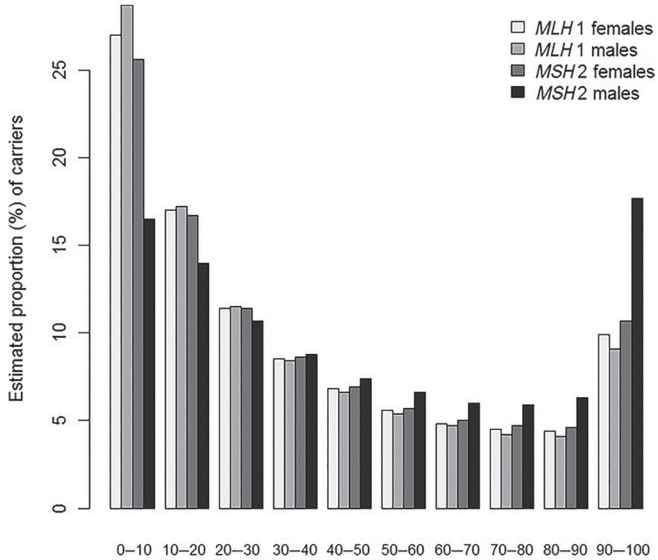


Figure 2 Cumulative cancer risks shows large variation between individuals with a germline pathogenic variant in MLH1 and MSH2.[42] Reprinted with permission of John Wiley and Sons.

antigen based vaccinations have been suggested as potential preventive measures in CMMRD, while treatment with immune checkpoint inhibitors can be effective once cancer has developed.[57,58] Another benefit of an early CMMRD diagnosis is the opportunity to counsel parents on the recurrence risk of 25% for future pregnancies; prenatal diagnostics and preimplantation genetic diagnostics are options that can be offered to parents who wish to have more children, but who want to prevent CMMRD from occurring in future offspring.

Despite its rarity, it may therefore be worthwhile to attempt at diagnosing CMMRD before the development of cancer. Although knowledge and recognition of the syndrome have increased over the years, it is likely that many patients are not diagnosed, particularly if they are a single case within a family and/or if they do not survive the first cancer (and therefore do not develop a second cancer that could raise suspicion of a cancer predisposition syndrome).

Challenges

Due to improved and early detection and removal of adenomas, the incidence of colorectal cancer is expected to decline with the recent introduction of population based screening for faecal blood.[59-62] While this is expected to have a positive effect on colorectal cancer morbidity and mortality,[61] this will mean there are less opportunities to identify Lynch syndrome patients through immunohistochemical staining of colorectal cancers. Immunohistochemical staining for the presence of the MMR proteins in adenomas is not as sensitive as staining of colorectal cancers as it has been shown that not all adenomas in Lynch syndrome patients show MMR deficiency.[63-65] Furthermore, immunohistochemical staining of large cohorts of adenomas resulted in very low MMR deficiency detection rates (0.3-0.4%).[66,67] To compensate for this decline in opportunities to identify carriers of a pathogenic MMR variant, other approaches can be explored. One approach could be the universal screening of cancers, other than colorectal and endometrial cancer, with a relatively high prevalence of Lynch syndrome associated MMR deficiency. One promising candidate for this approach would be small bowel cancer. While small bowel cancers are a relatively rare type of tumour, the prevalence of MMR deficiency in small bowel adenocarcinomas has been reported to be up to 35%, indicating that these tumours may be suitable candidates to perform universal MMR deficiency screening.[68,69] However, these estimations have been based on relatively small cohorts and show a wide range (5-35%),[69] which is why more research is needed to establish a more precise estimation. Furthermore, little is known about the prevalence of Lynch syndrome in these MMR deficient cases.

A second type of tumour with a relatively high penetrance in Lynch syndrome is ovarian cancer. While the association between Lynch syndrome and ovarian cancer has been well established, some discussion remains on the histology of MMR deficient ovarian cancers.[70,71] It has been suggested that standard (i.e. universal) screening for MMR deficiency in ovarian cancer should be limited to specific histological subtypes (*i.e.* endometrioid and clear-cell).[72] Arguing against this is a large meta-analysis which showed that, although less common than in endometrioid and clear-cell tumours, MMR deficiency is still present in 7.9% of high-grade serous ovarian cancers. In addition, 16.7-25% of ovarian cancers identified in Lynch syndrome patients are of high-grade serous histology.[73-75] Based on these numbers, a diagnosis of Lynch syndrome should still be considered when a patient seeks clinical genetic advice. Further research is required to help clinicians determine whether Lynch syndrome should be considered as a differential diagnosis in patients with high-grade serous ovarian cancer.

AIMS AND OUTLINE OF THIS THESIS

The aim of this thesis is 1) to provide insights that may help in the identification of patients with Lynch syndrome and CMMRD, and 2) to further elucidate the phenotype and potential modifying factors that result from carrying a germline pathogenic variant in one of the MMR genes. Both aims are important to further facilitate adequate detection and surveillance of individuals with a germline pathogenic variant in one of the MMR genes.

Part I The detection of patients with Lynch syndrome and CMMRD

In **chapter 2** the first case in literature is described where a diagnosis of CMMRD was made in a healthy child that presented with a neurofibromatosis-type-1-like phenotype. This case description initiated a discussion that resulted in a literature study and guidelines as described in **chapter 3** that indicate when clinicians should be testing for CMMRD in children with CALMs but without an *NF1* mutation. In **chapter 4** the frequency of MMR deficiency and Lynch syndrome in a large cohort of small bowel cancers is described and the implications of these findings for universal testing of MMR deficiency in these tumours are discussed. In **chapter 5**, a case series of serous ovarian cancers that were tested for MMR deficiency is presented and, combined with an overview of recent literature, it is discussed how these results impact on testing for Lynch syndrome in this group of cancer patients.

Part II Cancer penetrance in Lynch syndrome and potential factors of influence

In **chapter 6** a novel approach to estimating cancer risk in *PMS2*- and *MSH6*-associated Lynch syndrome is described. By analysing a large cohort of families where the index patient was diagnosed with CMMRD, the issue of ascertainment bias due to a positive family history is circumvented. In **chapter 7** the influence of genotype and parent-of-origin on the phenotype of *PMS2*-associated Lynch syndrome is analysed. In **chapter 8** the number of polyps and interval cancers in *PMS2* variant carriers is investigated and the implications of our findings in light of the relatively low cancer risks that have been reported for *PMS2* are discussed. In **chapter 9** the main findings of the previous chapters in relation to the most recent literature are discussed and suggestions are made on how to move forward with scientific research in the field of Lynch syndrome and CMMRD.

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