PMS2-associated Lynch syndrome : the odd one out

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ENGLISH SUMMARY

Colorectal cancer is one of the most frequently diagnosed cancers in the Western world. Both hereditary and genetic factors play a role in its etiology. In approximately 3% of colorectal cancer cases the underlying cause is a hereditary cancer predisposition syndrome called Lynch syndrome (formerly HNPCC, Hereditary Non-Polyposis Colorectal Cancer). This thesis focuses on an important subset of Lynch syndrome patients, namely those carrying a heterozygous pathogenic germline variant in the mismatch repair gene *PMS2*. Lynch syndrome can also be caused by pathogenic variants in one of the other mismatch repair genes *MLH1*, *MSH2* (and *EPCAM*) and *MSH6*. This condition gives an increased risk of developing mainly colorectal and endometrial cancer, but also – to a lesser extent - other forms of cancer.

Relatively little was known about PMS2-associated Lynch syndrome compared to Lynch syndrome caused by other genes. More research on the PMS2-associated Lynch syndrome was necessary because many questions still existed about this condition, such as the risk of developing cancer and the tumorigenesis. A further delineation of the PMS2-associated phenotype is important as the prevalence of *PMS2* variants has been largely underestimated due to stringent selection criteria and challenges in molecular diagnostics due to the existence of multiple pseudogenes. The aim of this thesis is to further define the phenotype associated with a pathogenic variant in the *PMS2* gene, and to work towards gene-specific clinical guidelines. It also describes studies of several external and internal risk modifiers in an attempt to explain the large clinical variation between and within families with *PMS2* variants.

Chapter 1 is an introduction on hereditary colorectal cancer and Lynch syndrome. The first step in the delineation of the PMS2 phenotype is described in **Chapter 2** and includes two studies reporting on the penetrance of pathogenic germline variants in the *PMS2* gene. In the first study, published in 2015, we analyzed 98 European families and reported cumulative risk up to age 70 of 11-19% for colorectal and 12% for endometrial cancer. This confirmed an earlier report from the United States that also stated that the penetrance for *PMS2* variants is significantly lower when compared to other MMR variants, where risks are reported to be up to 25-75%. Our study was the first to attempt risk analysis for other tumor locations associated with PMS2-associated Lynch syndrome as well. Unfortunately this study was limited by the cohort size. After further expansion of the cohort with families from the United States, Australia and Canada we set out to analyze these other cancers in more detail and with proper statistical methods to correct for ascertainment bias. In this second penetrance

analysis we reconfirmed the relatively low risk of colorectal and endometrial cancer, but also found that *PMS2* carriers were not at increased risk of any of the other Lynch-associated cancers, such as ovarian and gastric cancer.

While it is generally accepted that *PMS2* carriers face markedly lower penetrance, a high degree of phenotype variability is observed both within and between families. **Chapter 3** therefore includes three studies aimed at studying risk modifiers previously associated with Lynch syndrome. No risk modifying effects were identified when analyzing single nucleotide polymorphisms (SNPs) selected from Genome Wide Association Studies (GWAS) in sporadic colorectal cancer cohorts. This includes two loci at 11q23.1 and 8q23.3 that have been repeatedly associated with increased risk in (female) MLH1 carriers. We also attempted to estimate the effect of lifestyle factors (smoking, alcohol use and BMI at age 20) in a retrospective study. No clear effects were identified, although it should be noted that this study was relatively small (n=270 *PMS2* carriers) and may have lacked power. Lastly, a genetic anticipation study was undertaken. The existence of this phenomenon has been much debated. While we initially confirmed an anticipation effect of decreasing age at colorectal cancer, we show with the introduction of a new flexible Cox regression model that this effect is completely explained by a birth cohort effect. Thus, anticipation in PMS2 families seems to be due to a general trend in the general population rather than a biological effect that would increase risk with each subsequent generation in which the PMS2 variant is transmitted.

The notably different phenotype of PMS2-associated Lynch syndrome has led to speculation of differences in tumorigenesis. **Chapter 4** describes the role of PMS2 deficiency in tumorigenesis. This chapter includes two papers. The first is a collaboration with the University of Heidelberg on the presence of instability in coding microsatellites (cMS). The results showed that there are no clear differences in cMS profiles between PMS2 and MLH1/MSH2 tumors. This means that it is likely that similar neo-antigens appear on the celmembrane, which in turn makes it probable that vaccines now targeted at these neo-antigens might work for *PMS2* carriers as well. This chapter also describes the analysis of the somatic variant spectrum in PMS2-associated colorectal cancer. The most notable result was a complete lack of *CTNNB1* (β-catenin) variants in PMS2 tumors (0/20) while the majority of a control cohort of 24 MLH1 tumors did harbor such a variant (14/24). It has been suggested that *CTNNB1* mutated colorectal cancers actually represent an aggressive subtype of cancer which presents as post-colonoscopy colorectal cancers, i.e. cancers that arise in between follow-up colonoscopies. These in turn may be derived from mismatch repair deficient (dMMR) crypts that progress to colorectal cancer without a MMR proficient precursor

adenoma stage. Our data suggests that *PMS2* carriers do not develop colorectal cancer through dMMR crypts, thus explaining lower penetrance and the reported lack of post-colonoscopy colorectal cancers in prospectively observed cohorts. The latter observation is in contrast to other Lynch syndrome patients, where the cumulative risk of developing colorectal cancer is still up to 46% despite undergoing regular colonoscopies and polypectomies.

Chapter 5 investigates this further and contains a study describing prospectively collected data on PMS2 families. This data showed that high-grade adenomas were only rarely found and moreover that only two PMS2 carriers undergoing surveillance developed post-colonoscopy colorectal cancers in over a 1000 years of observation. The latter might be seen as conflicting in light of our hypothesis of a lack of tumor development from dMMR crypts. However upon closer inspection of the colonoscopy reports, the preceding colonoscopies proved unreliable due to insufficient cleansing of the colon and obesity. Moreover, one of these tumors was MMR proficient and it was therefore unlikely a consequence of the underlying germline *PMS2* variant. The last relevant observation in this study is that immunohistochemistry of 16 low-grade adenomas showed no abrogation of the PMS2 protein. Moreover, the proportion of carriers free from adenomas was much higher for *PMS2* carriers when compared to *MLH1* and *MSH2* carriers. This confirms the notion that PMS2 deficiency only occurs at a later stage of colorectal cancer development, rather than being the initiating factor as *MLH1/MSH2* carriers may also form dMMR adenomas from dMMR crypts.

In summary, this thesis shows that *PMS2* carriers are a separate entity among Lynch syndrome patients. This is based on both epidemiological analyses of the clinical phenotype and on tumor analyses. Following the results of the studies in this thesis and studies by other groups, we have formulated preliminary PMS2-specific surveillance guidelines. This entails that *PMS2* carriers start with 2-3 yearly colonoscopies beginning at age 35-40. There seems to be no role for risk-reducing gynecological surgery given the low risk of endometrial cancer with a good chance of survival. Routine transvaginal ultrasounds with biopsies every 1-2 years from age 40-60 can be considered, but female carriers should be properly informed that there is currently no conclusive evidence for better survival after early detection of endometrial cancer.

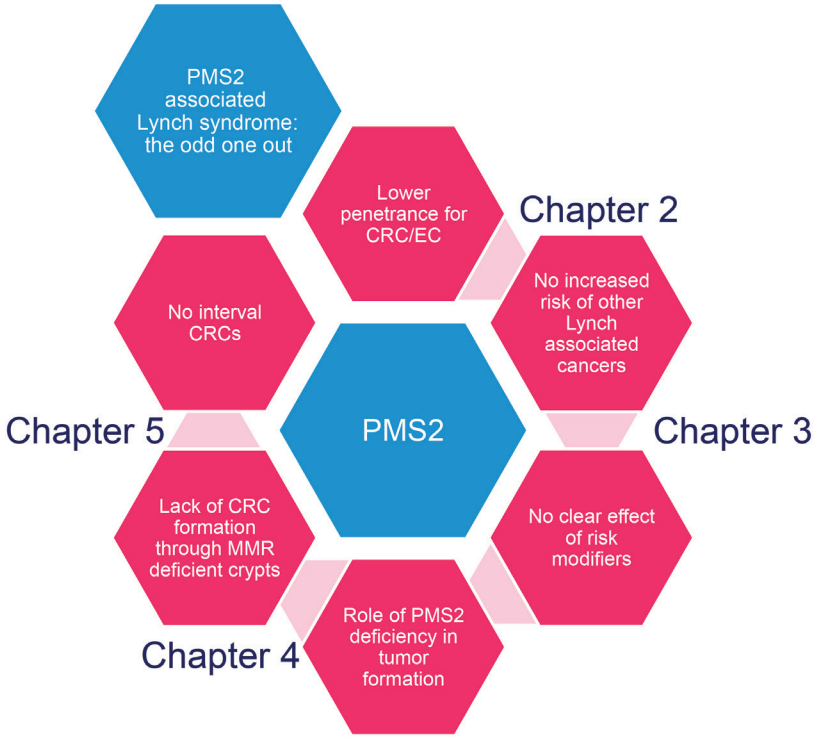

FIGURE 1 Overview of thesis