PMS2-associated Lynch syndrome : the odd one out

Broeke, S.W. ten

3.2

The risk modifying effect of lifestyle factors in patients with PMS2-associated Lynch syndrome

Manuscript in preparation

Sanne W. ten Broeke, Ghazaleh Dashti, Encarna Gomez Garcia, Liselot P. van Hest, Tom G.W. Letteboer, Lizet E. van der Kolk, Maran J.W. Olderode-Berends, Theo A. van Os, Liesbeth Spruijt, Anja Wagner, Ellen Kampman, Franzel van Duijnhoven, Aung Ko Win, Maartje Nielsen

ABSTRACT

Introduction

The clinical phenotype of *MLH1*, *MSH2*, *MSH6* has been thoroughly described. There are, however, still many outstanding questions concerning the clinical phenotype of Lynch Syndrome patients with a *PMS2* variant. Variants in the *PMS2* gene display a lower penetrance compared to *MLH1* and *MSH2* for cancer, and a wide interfamilial variance in clinical phenotype. It is therefore likely that external factors or genetic modifiers are involved in the *PMS2* phenotype. The aim of this retrospective study was to assess whether lifestyle factors influence colorectal cancer risk in this subset of Lynch patients.

Methods

To assess whether lifestyle factors influence colon cancer risk and polyp count, lifestyle questionnaires were sent to 193 *PMS2* carriers. This questionnaire was developed by investigators of Leiden University and Wageningen University and included 7 questions about: sex, height, weight, smoking, alcohol and lastly whether they had used aspirin. Additional data was collected in collaboration with the Colon Cancer Family Registry (CCFR). A weighted cox-proportional hazards regression model was used to estimate hazard ratios.

Results

A total of 270 *PMS2* carriers were included. There was no evidence of a strong association between BMI at age 20, smoking or alcohol consumption and the risk of colorectal cancer. Of note, a possible trend was observed for *PMS2* carriers that were overweight/obese at age 20 (HR 1.32, 95% CI: 0.59-2.97, p= 0.09).

Conclusion

In summary, we found no strong association of smoking, obesity in adolescence, or alcohol use with colorectal cancer risk in our cohort of 270 PMS2-associated Lynch syndrome patients. Future studies should have a prospective design and focus an adenoma occurrence as an endpoint.

INTRODUCTION

Patients with Lynch syndrome have a hereditary predisposition for the development of colorectal cancer, endometrial cancer and several other cancers. These patients carry a heterozygous pathogenic germline variant in one of the mismatch repair (MMR) genes; *MLH1*, *MSH2*, *MSH6* or *PMS2*, or a deletion in *EPCAM* which causes dysfunction of *MSH2*. A second somatic hit results in malfunctioning of the MMR system, which in turn leads to the accumulation of somatic variants in other genes and can ultimately result in cancer. The reported cancer risk varies widely[1] and appears to differ not only between families but also between members of the same family.[2] Multiple theories have been proposed to explain this phenomenon, which include the possible risk modifying effect of lifestyle factors such as cigarette smoking, obesity or alcohol consumption.[3] These factors may have a different effect on the development of colorectal cancer in Lynch syndrome patients, primarily because of the differences in tumorigenesis compared to sporadic colorectal cancer cases. Indeed, it has been suggested that hereditary cancer patients might be more susceptible for lifestyle factors.[3] This increased effect in hereditary colorectal cancer could be due to the fact that only a second hit is needed for a defect in the MMR machinery[4] where sporadic microsatellite instable tumors need two somatic hits within the same MMR gene. In other words, damaging lifestyle factors might increase the likelihood of a second hit in the wild type allele of MMR germline mutated patients.

Campbell et al. found evidence that recent BMI and adult weight gain were associated with the microsatellite stable phenotype of colorectal cancer cases.[5] In line with this finding, Win et al also linked obesity (at age 20 years) to colorectal cancer, but found no difference in the increase in risk of colorectal cancer between carriers and non-carriers.[6] The same group also investigated the effect of BMI on endometrial cancer. Interestingly, only non-MMR mutation carriers were found to be at increased risk at higher BMI, suggesting that other pathways besides the estrogen pathway are important in endometrial carcinogenesis in female Lynch patients.[7] A large study by Pande et al. showed statistically significant increased hazard ratios of colorectal cancer for Lynch patients that smoked cigarettes.[8] However this study did not include patients that carry a variant in *PMS2*.

The cancer risk for *PMS2* carriers is lower compared to carriers of a variant in one of the other MMR genes[9, 10] and recent studies have suggested differences in tumorigenesis which may also result in a different effect of lifestyle factors in this specific subset of Lynch patients.[11] In this study we used a case-control design to investigate the effect

of lifestyle factors on the development of colorectal cancer and endometrial cancer in a combined Dutch, Australasian and Northern-American cohort of 270 *PMS2* carriers.

METHODS

Data collection

Dutch cohort

Available pedigree and patient specific data has been collected from 2009 until 2015 in collaboration with the clinical genetic departments of the university hospitals in the Netherlands. We received informed consent of 193 Dutch *PMS2* carriers. Patient records were screened in an attempt to confirm all clinical and pathological data where possible. Most index patients (probands) were sent in for variant analysis because their phenotype and/or the family history was suspect for Lynch Syndrome. Some people also gave consent to use data on their deceased relatives. To decrease the risk of survival bias, we approached these family members to also fill in a lifestyle questionnaire on their deceased relative. Excluding these cases would mean that carriers that die at a young age (e.g. from a Lynch syndrome associated cancer) are not included in the analyses and could thereby lead to a decrease in the total effect on the outcome measure, i.e. a bias towards null.

All confirmed carriers of *PMS2* variants with informed consent were sent a questionnaire on lifestyle factors. This questionnaire was developed by investigators of Leiden University and Wageningen University and included 7 questions about: sex, height, weight (at age 18 and age 40 years if applicable), smoking (duration, number of cigarettes and when applicable year of cessation), alcohol (duration, number of units per week) and lastly whether they had used aspirin. Carriers were asked specifically for their use just before they were diagnosed with cancer, polyps or upon entering screening. The response rate was 81%.

Colon Cancer Family Registry

Data collection from the Colon Cancer Family Registry (CCFR) has been described previously by Newcomb et al.[12,] and at www.coloncfr.org. In brief, data was collected between 1998 and 2012. The CCFR recruited families through population-based probands that were diagnosed with colorectal cancer. These families originate from the USA (Washington, California, Arizona, Minnesota, Colorado, New Hampshire, North Carolina, and Hawaii), Australia (Victoria) and Canada (Ontario). Clinically ascertained probands were also included and were derived from families referred to family cancer

clinics in the USA (Mayo Clinic, Rochester, Minnesota, and Cleveland Clinic, Cleveland, Ohio), Canada (Ontario), Australia (Melbourne, Adelaide, Perth, Brisbane, Sydney and Newcastle) and New Zealand (Auckland). The process of obtaining informed consent is outlined in Newcomb et al.[12] The study protocol was approved by the institutional research ethics review board of which the family members were derived. Clinical data was obtained through extensive questionnaires. The total cohort of which lifestyle data was available included 100 participants.

Statistical analysis

Previous studies have described the oversampling of cases in clinic-based cohorts, which is the larger part of the currently analyzed cohort. These carriers usually belong to high-risk families, ascertained because of their relatively severe phenotype. Moreover affected family members are more likely to be tested for the variant and this too gives over-sampling of cases. To account for this bias, we used a weighted cohort approach, previously described by Antoniou et al.[13] Weights were calculated based on incidence rates from either the Dutch or the American population (for CCFR patients). Hazard ratios (HRs) based on a proportion of this cohort were previously reported and used to determine age stratum (5 year) specific weights.[10] All calculated weights for cases were smaller than 1, effectively down-weighting cases compared with controls. It is important to note that for hypothesis testing the unweighted p-value and confidence interval are to be used.

A Cox-proportional hazards regression model was used to estimate HRs. The time at risk for every participant was set at age 20 and ended at the age of diagnosis of colorectal cancer (n=93), age of diagnosis of any other cancer (n=42), or age at interview (n=133), whichever occurred first. The rationale behind censoring for other cancers is that this affects the risk of developing colorectal cancer, for example due to (long-term) treatment effects. Estimates were corrected for familial clustering of risk by using the Huber-White sandwich estimator. Some variables were analyzed as time-dependent covariates because not doing so resulted in a violation of the proportional hazards assumption, which was investigated by examining the Schoenfeld residuals with a formal statistical test and by plotting them against time. Time-varying variables were generated for polypectomy, smoking status, and total pack-years smoked by splitting dataset per year and taking into account age at first polypectomy, age at initiation of smoking, age at quitting smoking, and years of smoking. Due to the retrospective design of this study taking into account recent BMI is difficult, therefore we used BMI in early adulthood (18-20 years) as a covariate in the model.

All analysis were performed in Stata, version 20.

RESULTS

A total of 270 *PMS2* carriers were included, results are given in table 1. There was no evidence of a strong association between BMI at age 20, smoking or alcohol consumption and the risk of colorectal cancer. Of note, a possible trend was observed for *PMS2* carriers that were overweight/obese at age 20 (HR 1.32, 95% CI: 0.93-2.83, p= 0.09).

TABLE 1 Hazard ratios for associations between sex, BMI at age 20, polypectomy, smoking, and alcohol consumption and the risk of colorectal cancer for participants with a germline variant in *PMS2*.

			Unweighted univariable model		Weighted univariable model	
	No. with CRC	Person-years	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Sex						
Female	43	5064	1 [Reference]		1 [Reference]	
Male	50	3422	1.65 (1.10 – 2.46)	0.02	2.14 (1.21 – 3.77)	0.01
BMI at age 20						
Normal	68	6324	1 [Reference]		1 [Reference]	
Underweight	6	862	0.69 (0.33 – 1.43)	0.32	0.44 (0.16 – 1.21)	0.11
Overweight/ Obese	16	1098	1.63 (0.93 – 2.83)	0.09	1.32 (0.59 – 2.97)	0.5
Any polypectomy						
No	90	8228	1 [Reference]		1 [Reference]	
Yes	3	258	0.84 (0.28 – 2.53)	0.76	0.81 (0.22 – 2.99)	0.75
Smoking status						
Never	39	3281	1 [Reference]		1 [Reference]	
Former	31	2002	0.93 (0.57 – 1.52)	0.78	0.81 (0.38 – 1.70)	0.58
Current	23	3203	0.81 (0.48 – 1.35)	0.42	0.89 (0.43 – 1.84)	0.74
Total pack-year*	62	6762	1.00 (0.97 – 1.03)	0.93	1.01 (0.97 – 1.05)	0.62
Alcohol consumption (units/week)	53	6218	0.97 (0.93 – 1.01)	0.16	0.95 (0.90 – 1.00)	0.07

*Pack-years: Number of cigarettes per day multiplied by the number of years

DISCUSSION

In this study we investigated the association of BMI, smoking and alcohol consumption with the development of colorectal cancer in Lynch syndrome patients carrying a *PMS2* variant. Notably we did not see major effects of any of these lifestyle factors. Interestingly, we did observe a trend towards carriers with overweight in adolescence being at increased risk of colorectal cancer, which is in line with previous observations by Win et al.[14] It should be noted however that previous studies have linked obesity to MSS tumors, whereas Lynch-associated colorectal cancers are usually MSI-high.[5] Indeed, the previous study by Win et al. found no significant difference between MMR carriers compared to non-carriers, which suggests that the effect is not stronger in Lynch syndrome, but similar to the general population.[14] Clinical interference on BMI at adolescence if of course problematic as many carriers only become apparent after age 20, even those that are tested pre-symptomatically, and preventive weight loss might be too late as the damage could have already been done. Therefore advises concerning weight should also focus on children of known carriers, even before variant screening is performed.

In contrast to BMI, smoking has previously been associated with MSI-H colorectal cancer. Paradoxically, cigarette smoking appeared to be associated with colorectal adenomas in most studies, but reports are inconsistent about the association between cigarette smoking and colorectal cancer.[3, 8, 15-18] This is however, most likely due to the fact that cigarette smoking only contributes to the development of a minority of colon cancers, namely those that are MSI-H.[17] This makes it a particularly interesting potential risk modifier in Lynch syndrome, as MSI is a known hallmark of Lynch associated colorectal cancer. Slattery et al. estimated that 21% of MSI in colon tumor tissue might be attributed to cigarette smoking.[16] The biological explanation for this could be that smoking cigarettes causes replication errors in too large a number to be repaired by the MMR system or that it interferes within the MMR system itself. In patients with Lynch syndrome with an already vulnerable MMR system the latter might lead to higher risk of colorectal cancer. Indeed, a previous study reported significant HRs for MMR mutation carriers. This study reported a significant HR (2.15 (1.22-3.8)) for the heterodimer partner PMS2, MLH1.[8] However, we were not able to confirm the risk modifying effects of smoking in this PMS2 cohort. This could have several biological reasons. One explanation could be that the effect of smoking in Lynch syndrome is gene-specific. MLH1 and PMS2 proteins function in a heterodimer within the MMR machinery, as do MSH2 and MSH6. Notably, MLH1 can also form a heterodimer with MLH3 or PMS1, which might in part explain both the lower penetrance but also a

lower damaging effect of smoking, because there is still a partly functioning MMR system even in the absence of PMS2.[19] Conversely, the same study as mentioned above also reported a significantly increased HR (6.02 (1.40-25.87)) for *MSH6* carriers, while the MSH2 protein also has the capacity to bind with another protein, namely MSH3. Hence, this does not seem to be the final answer.[8] Another possibility is that *PMS2* is less susceptible for a second hit caused by lifestyle factors, compared to for example *MSH6*. Indeed, the *MSH6* gene is known to have a microsatellite region which may be a potential target for smoke-related DNA toxicity. This second hit theory has been suggested as an explanation for a higher susceptibility to lifestyle mediated cancer risk increase in hereditary cancer patients.[4] More functionally oriented studies are needed to be able to investigate the underlying biological mechanism further. Lastly, lack of an association might also be caused by (genetic) heterogeneity of the cohort, for example interaction of smoking with SNPs in xenobiotic metabolizing enzymes such as CYP1A1.[3] This might mean that only a proportion of (*PMS2*) carriers is at an increased risk when they smoke, which might have been missed in this crude analysis. Identifying these cases could select carriers that should be counselled more proactively for smoking cessation.

It should be noted however, that the lack of significant results in this study could also be a consequence of a relatively small cohort size, which is a limitation of this study. Another limitation is the retrospective case-control design, which means that there is a chance of selection bias. We attempted to minimize this bias by using a weighted cohort approach. Lastly, the parameters investigated in this study were self-reported which means that recall bias could be present. This is especially relevant for questionnaires of deceased carriers that were filled in by family members. Conversely, these questionnaires are also a strength of this study, as they limit survival bias, i.e. we do not exclude people with a poor clinical outcome.

In summary, we found no strong association of smoking, obesity in adolescence, or alcohol use with colorectal cancer risk in our cohort of 270 PMS2-associated Lynch syndrome patients, although there might be a trend for those *PMS2* carriers that are obese in adolescence. Larger studies are needed to investigate this finding further. As recent work by our group suggested that colorectal cancer in *PMS2* carriers may only develop through the adenoma-to-colorectal cancer pathway (ten Broeke et al, 2018, accepted at Gastroenterology), using the development of adenomas as an endpoint might be of particular interest in this subset of Lynch patients. Future studies should therefore focus on prospectively obtained data.

3

REFERENCES

1. Barrow E, Hill J, Evans DG. Cancer risk in Lynch Syndrome. Fam.Cancer 2013;12:229-240.
2. Dowty JG, Win AK, Buchanan DD, et al. Cancer risks for MLH1 and MSH2 mutation carriers. Hum.Mutat. 2013;34:490-497.
3. Van Duijnhoven FJ, Botma A, Winkels R, et al. Do lifestyle factors influence colorectal cancer risk in Lynch syndrome? Fam.Cancer 2013;12:285-293.
4. Botma A, Vasen HF, van Duijnhoven FJ, et al. Dietary patterns and colorectal adenomas in Lynch syndrome: the GEOLynch cohort study. Cancer 2013;119:512-521.
5. Campbell PT, Jacobs ET, Ulrich CM, et al. Case-control study of overweight, obesity, and colorectal cancer risk, overall and by tumor microsatellite instability status. J.Natl.Cancer Inst. 2010;102:391-400.
6. Win AK, Dowty JG, English DR, et al. Body mass index in early adulthood and colorectal cancer risk for carriers and non-carriers of germline mutations in DNA mismatch repair genes. Br J Cancer 2011;105:162-9.
7. Win AK, Dowty JG, Antill YC, et al. Body mass index in early adulthood and endometrial cancer risk for mismatch repair gene mutation carriers. Obstet. Gynecol. 2011;117:899-905.
8. Pande M, Lynch PM, Hopper JL, et al. Smoking and colorectal cancer in Lynch syndrome: results from the Colon Cancer Family Registry and the University of Texas M.D. Anderson Cancer Center. Clin.Cancer Res. 2010;16:1331-1339.
9. Senter L, Clendenning M, Sotamaa K, et al. The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. Gastroenterology 2008;135:419-428.
10. ten Broeke SW, Brohet RM, Tops CM, et al. Lynch syndrome caused by germline PMS2 mutations: delineating the cancer risk. J Clin Oncol 2015;33:319-25.
11. Alpert L, Pai RK, Srivastava A, et al. Colorectal Carcinomas With Isolated Loss of PMS2 Staining by Immunohistochemistry. Arch Pathol Lab Med 2018.
12. Newcomb PA, Baron J, Cotterchio M, et al. Colon Cancer Family Registry: an international resource for studies of the genetic epidemiology of colon cancer. Cancer Epidemiol Biomarkers Prev 2007;16:2331-43.
13. Antoniou AC, Goldgar DE, Andrieu N, et al. A weighted cohort approach for analysing factors modifying disease risks in carriers of high-risk susceptibility genes. Genet.Epidemiol. 2005;29:1-11.

14. Win AK, Dowty JG, English DR, et al. Body mass index in early adulthood and colorectal cancer risk for carriers and non-carriers of germline mutations in DNA mismatch repair genes. Br.J.Cancer 2011;105:162-169.
15. Limsui D, Vierkant RA, Tillmans LS, et al. Cigarette smoking and colorectal cancer risk by molecularly defined subtypes. J Natl Cancer Inst 2010;102:1012-22.
16. Slattery ML, Curtin K, Anderson K, et al. Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors. J.Natl.Cancer Inst. 2000;92:1831-1836.
17. Watson P, Ashwathnarayan R, Lynch HT, et al. Tobacco use and increased colorectal cancer risk in patients with hereditary nonpolyposis colorectal cancer (Lynch syndrome). Arch.Intern.Med. 2004;164:2429-2431.
18. Winkels RM, Botma A, Van Duijnhoven FJ, et al. Smoking increases the risk for colorectal adenomas in patients with Lynch syndrome. Gastroenterology 2012;142:241-247.
19. Peltomaki P. Update on Lynch syndrome genomics. Fam Cancer 2016.