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RESEARCH ARTICLE

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Delineating genotype and parent-of-origin effect on the phenotype in *MSH6*-associated Lynch syndrome

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Abstract

Background: This study investigates the potential influence of genotype and parent-of-origin effects (POE) on the clinical manifestations of Lynch syndrome (LS) within families carrying (likely) disease-causing *MSH6* germline variants.

Patients and Methods: A cohort of 1615 *MSH6* variant carriers (310 LS families) was analyzed. Participants were categorized based on RNA expression and parental inheritance of the variant. Hazard ratios (HRs) were calculated using weighted Cox regression, considering external information to address ascertainment bias. The findings were cross-validated using the Prospective Lynch Syndrome Database (PLSD) for endometrial cancer (EC).

Results: No significant association was observed between genotype and colorectal cancer (CRC) risk (HR = 1.06, 95% confidence interval [CI]: 0.77–1.46). Patients lacking expected RNA expression exhibited a reduced risk of EC (Reference Cohort 1: HR = 0.68, 95% CI: 0.43–1.03; Reference Cohort 2: HR = 0.63, 95% CI: 0.46–0.87).

Abbreviations: CRC, colorectal cancer; EC, endometrial cancer; HR, hazard ratio; LS, Lynch syndrome; *MLH1*, MutL homolog 1; MMR, mismatch repair; *MSH2*, MutS homolog 2; *MSH6*, MutS homolog 6; NMD, nonsense-mediated decay; PLSD, Prospective Lynch Syndrome Database; *PMS2*, post-meiotic segregation increased 2; POE, parent of origin.

However, these results could not be confirmed in the PLSD. Moreover, no association was found between POE and CRC risk (HR = 0.78, 95% CI: 0.52–1.17) or EC risk (Reference Cohort 1: HR = 0.93, 95% CI: 0.65–1.33; Reference Cohort 2: HR = 0.8, 95% CI: 0.64–1.19).

Discussion and Conclusion: No evidence of POE was detected in *MSH6* families. While RNA expression may be linked to varying risks of EC, further investigation is required to explore this observation.

KEYWORDS
cancer risks, colorectal carcinoma, endometrial carcinoma, Lynch syndrome

1 | INTRODUCTION

Lynch syndrome (LS) (OMIM: 614350) is caused by a (likely) disease-causing variant in one of the mismatch repair (MMR) genes, which include *MLH1*, *MSH2*, *MSH6*, or *PMS2*.[1] LS is characterized by clustering of colorectal cancer (CRC) and endometrial cancer (EC) and is also associated with an increased risk of cancers of the ovaries, upper urinary tract, upper gastrointestinal tract, brain, sarcoma, and prostate.[2]

As our understanding of affected families and patients has improved, it has become increasingly clear that cancer risk varies not only by gene and gender,[2–5] but also between and within affected families with disease-causing variants in the same gene.[6] This phenotypic variation is most likely due to environmental factors, genetic factors, or a combination of the two. Genotype–phenotype correlations and parent-of-origin effects (POE) have also been suggested as potential causes for interfamilial[5–10] and intrafamilial variance.[11–14] However, conflicting results have been reported.

Although genotype–phenotype correlations and POE have been explored in several studies, *MSH6* families are not well represented. Therefore, this study aimed to analyze whether POE or genotype–phenotype correlations explain the intra- and interfamilial cancer risk variance seen in *MSH6* variant carriers. If so, these two factors may be important in the clinical management of *MSH6* variant carriers.

2 | PATIENTS AND METHODS

2.1 | Cohort description—Dutch cohort

This research was approved by the LUMC Ethics Review Board (P17.098). Data on *MSH6*-associated LS families counseled up to March 2021, were collected at the following Dutch clinical genetics departments: Amsterdam Medical Center, VU Medical Center, Netherlands Cancer Institute, Erasmus MC Cancer Institute, Leiden University Medical Center, Maastricht University Medical Center, University Medical Center Utrecht, and University Medical Center Groningen. Most families were clinic-based and fulfilled the (revised) Bethesda criteria,[15] although some population-based families were detected by universal tumor screening.[16] Obligate carriers and proven heterozygous (likely) disease-causing variant carriers were included in the study. Informed consent was obtained from patients by their respective genetic counselors. Cancer diagnoses were verified for consenting patients. Patients under 18 years were excluded as no phenotype was expected before this age. Patients with additional class 3–5 MMR or *MUTYH* variants were also excluded due to the possible influence on cancer risk.[17] In 12 patients, tumors also showed negative or weak staining of the MSH2 protein. No germline *MSH2* variant was identified, and in cases where tumor reanalysis was feasible, no explanatory MSH3 variants were found, consistent with previous findings reported by our research group.[18] The *MSH6* variants in this study were detected as part of the clinical genetic diagnostic procedure. An overview of the included variants can be found in Table S1.

2.2 | Cohort description—Prospective Lynch Syndrome Database cohort

The Prospective Lynch Syndrome Database (PLSD) is a prospective observational study without a control group that was designed in 2012. It provides an aggregated compilation of combined genetic and clinical information from 8500 carriers of (likely) disease-causing MMR variants, with a total follow-up of 71 713 years.[19] The PLSD was used as a replication cohort.

2.3 | RNA analysis

MSH6 (likely) disease-causing variants, primarily nonsense and frameshift variants, were subgrouped based on a prediction of RNA expression or, in some cases, known RNA expression status:

- Subgroup 1: Expected or known RNA expression
- Subgroup 2: No RNA expression expected or known
- Subgroup 3: Unknown RNA expression

Classification of RNA expression was carried out as recommended by Inácio et al.[20] and Shyu et al.,[21] or if clinical data on RNA expression were available. Briefly, nonsense-mediated decay (NMD) was predicted for truncating variants occurring before or in the

second-to-last exon, with the stop occurring 50 nucleotides before the splice donor site of the second-to-last codon. In the case of the *MSH6* gene, this implies that a stop after codon 1317 is unlikely to result in NMD. Patients with variants with unknown RNA expression were excluded from the analysis ($n = 42$).

2.4 | Statistics

In the Dutch cohort, data for the CRC and EC risk analyses were censored by last known age or age of death, age of cancer diagnosis, age of first polypectomy or the start of colonic screening (in case of censoring for CRC), and hysterectomy (in case of censoring for EC), whichever came first. Missing ages at cancer diagnosis or last known age were imputed using the mean for that specific cancer, allowing for the inclusion of as many family members as possible. Descriptive analysis included displaying Kaplan–Meier curves and conducting log rank tests to compare age at CRC and EC onset between the subgroups defined by RNA expression (Subgroup 1 vs. Subgroup 2) and POE (father vs. mother). The chi-square test was used to compare differences in terms of sex, cancer status at the end of follow-up, and POE among RNA expression subgroups. Descriptive statistics and analyses were performed using the IBM SPSS Statistics X20 package, with a $p < 0.05$ considered statistically significant.

Inverse probability of selection weighted Cox models[22] were fitted in R to correct for potential ascertainment bias in the Dutch cohort. Weights were derived using external population data (age-specific cumulative incidence rates) from the International Mismatch Repair Consortium[6] (CRC) or Baglietto et al.[23] and Dominguez-Valentin et al.[2] for EC.

A different approach was used for the PLSD cohort. Since the PLSD cohort is a prospective study, no correction for ascertainment bias was implemented. Sex- and age-specific cumulative incidence rates with 95% pointwise Poisson confidence intervals were calculated for each subgroup under investigation. All Dutch contributions to the PLSD were excluded to avoid duplication. Patients were similarly subgrouped as described above.

3 | RESULTS

The total cohort consisted of 1615 individuals from 316 families, of whom 709 were male (43.9%). CRC was diagnosed in 375 cases and EC in 212 patients. A total of 78 unique variants were included, with c.651dupT, p.(Lys218*) being the most common (16.4%), followed by c.467C>G, p.(Ser156*) (15.5%).

3.1 | Genotype–phenotype association

Among the variant carriers, RNA expression was expected in 117 cases (Subgroup 1), while no RNA expression was expected in 1498 cases (Subgroup 2). An overview of cohort characteristics is provided in Table 1. The log rank test did not show statistically significant differences in the crude age distribution at CRC between RNA expression subgroups ($p = 0.59$). However, a statistically significant difference was observed in the crude distribution of age at EC ($p = 0.007$). Kaplan–Meier curves are depicted in Figures S1 and S2.

In Subgroup 1, 24 patients developed CRC compared to 351 in Subgroup 2. When Subgroup 1 is compared to Subgroup 2 using weighted Cox regression, no significant association was observed for genotype–phenotype correlation regarding CRC risk (hazard ratio [HR] = 1.06 [95% confidence interval (CI): 0.77–1.46]).

EC was diagnosed in 21 and 191 females in Subgroups 1 and 2, respectively. Weighted Cox regression using Baglietto et al.[23] as a reference, comparing Subgroup 1 with Subgroup 2 indicated a lower EC risk in Subgroup 2 (no RNA expression) (HR = 0.68 [95% CI: 0.43–1.03]), as shown in Table 2. However, this association did not reach statistical significance. A limitation of Baglietto et al.[23] they do not provide age-specific cancer risks below 50 years of age. To overcome this potential limitation, the analysis was repeated using the data published in Dominguez-Valentin et al.,[2] which provides more data for young ages. The same trend was observed with slightly less uncertainty (HR = 0.63 [95% CI: 0.46–0.87]).

3.1.1 | PLSD cohort

Subgroup 1 (with [expected] RNA expression) contains 667 follow-up years, including 8 women diagnosed with EC. In Subgroup 2 (with [expected] no RNA expression), 35 women were diagnosed in 2914 follow-up years. Cumulative incidences are depicted in Figure S3.

3.2 | Parent-of-origin effect

In 1035 of 1615 patients, the parent of origin is known. Among them, 159 patients were diagnosed with CRC, and 85 females were diagnosed with EC (Table 2). The log rank test did not show statistically

TABLE 1 Overview of RNA subgroups.

	RNA subgroups	
	RNA expression expected ($n = 117$)	No RNA expression expected ($n = 1498$)
Sex		
Male (%)	48.7	43.6
Female (%)	51.3	56.0
Unknown (%)	0	0.5
Cancer		
CRC (%)	20.5 ($n = 24$)	23.4 ($n = 351$)
EC (% of females)	35.0 ($n = 21$)	22.8 ($n = 191$)
Age of diagnosis (mean and range)		
CRC (age range)	56.2 (24–76)	55.9 (26–84)
EC (age range)	53.4 (36–71)	55.8 (31–86)

significant differences in the crude age distribution at CRC ($p = 0.186$). However, a significant difference was observed for EC ($p = 0.001$). See also Figures S4 and S5.

Weighted Cox regression analysis revealed a lower risk of CRC (HR = 0.78 [95% CI: 0.52–1.17]) if the variant was paternally inherited, although this association was not statistically significant. Regarding EC, using Baglietto et al.[23] as a reference, the HR was 1.19 (95% CI: 0.65–2.40), while using Dominguez-Valentin et al.[2] as the reference, the HR was 0.87 (95% CI: 0.64–1.19). The wide 95% CI, especially in the case of Baglietto et al.[23] as the reference, indicates that no clear association between parent of origin and the risk of EC can be concluded (Table 3).

4 | DISCUSSION

This study focused on possible genotype–phenotype correlations and POE in carriers of *MSH6* (likely) disease-causing variants. Although previous studies have reported a POE in LS for *MSH6* and other MMR genes,[11,13] we did not find evidence of this effect. This is consistent with our previous study in LS patients with a disease-causing *PMS2* variant[14] and a recent study by Gemechu et al.[10]

Our analysis did not show a genotype–phenotype association for CRC. However, an association was found between genotype and EC risk, as patients carrying a variant associated with (expected) RNA expression had a higher risk for the development of EC. The pattern was observed using both reference cohorts, although statistical significance was achieved in only one of these analyses.[2] Interestingly, Ryan et al.[9] reported similar results for EC in *MLH1* variant carriers, although the same phenomenon was not observed in a *PMS2* cohort.[14]

An increased risk associated with predicted RNA expression may be attributed to a dominant-negative effect, as suggested by Ryan et al.[9] In the case of RNA expression, a (partially) functional protein is expected. The allele might therefore be (co)dominant to the wildtype, with the dysfunctional protein still participating in MSH2 binding, thereby causing genomic instability. Compared to an absent or non-functional MSH2 protein, a partially (dys)functional protein may therefore convey a higher cancer risk.[9,24] Similar dominant-negative effects have been described in other syndromes, such as ataxia telangiectasia and Coffin–Siris syndrome.[24,25]

To further explore if retention of RNA expression is associated with a higher risk for EC, we analyzed the risk in a reference cohort with *MSH6* (likely) disease-causing variant carriers from the PLSD database. This analysis did not show a genotype–phenotype correlation for EC, but unfortunately, only a few events of EC occurred in this cohort, making the outcome less reliable.

It should also be noted that the number of patients included in Subgroup 1 (expected RNA expression) was smaller than for Subgroup 2 (no RNA expected), which may have affected both the power and the results of this study. In addition, variants were subgrouped based on a prediction or known status of RNA expression, leading to the possibility of misclassification. For example, a stop codon after codon 1317 should result in stable RNA. Some variants in our cohort were close to codon 1317. Furthermore, confounders that have a proven influence on (colorectal) cancer development,[6] such as lifestyle, diet, aspirin use,[26–28] or low penetrant genetic risk modifiers,[29] were not

TABLE 2 Cohort overview parent of origin subgroups.

	Parent of origin subgroups	
	Maternally inherited ($n = 571$)	Paternally inherited ($n = 464$)
Sex		
Male (%)	41.7	49.4
Female (%)	58.3	50.6
Unknown (%)	0	0
Cancer		
CRC (%)	14.7 ($n = 84$)	16.2 ($n = 75$)
EC (% of females)	15.0 ($n = 50$)	14.9 ($n = 35$)
Age of diagnosis (mean and range)		
CRC (age range)	51.7 (28–79)	52.5 (26–81)
EC (age range)	55.7 (31–85)	53.2 (36–74)

TABLE 3 Hazard ratios for genotype effect and POE.

	Cancer	Comparison	HR of weighted analysis (95% CI)
Genotype–phenotype correlation	CRC	RNA expression vs. no RNA expression	1.06 (0.77–1.46)
	EC	RNA expression vs. no RNA expression	0.68 (0.43–1.03)[a]
	EC	RNA expression vs. no RNA expression	0.63 (0.46–0.87)[b]
Parent-of-origin effect	CRC	Paternally vs. maternally	0.78 (0.52–1.17)
	EC	Paternally vs. maternally	1.19 (0.65–2.40)[a]
	EC	Paternally vs. maternally	0.87 (0.64–1.19)[b]

Abbreviations: CI, confidence interval; CRC, colorectal cancer; EC, endometrial cancer; HR, hazard ratio; POE, parent-of-origin effects.
[a]Reference population of Baglietto et al.[23]
[b]Reference population of Dominguez-Valentin et al.[2]

taken into account. However, cultural and environmental differences were minimized by including almost all identified Dutch families with known (likely) disease-causing *MSH6* variants.

5 | CONCLUSION

Our findings indicate an association between retained RNA expression and a higher risk for EC, although this finding could not be confirmed in the PLSD cohort. No increased risk for CRC was observed in relation to retained RNA expression. These results should be interpreted with caution, as variant-specific risk factors may have a small but still important influence on cancer risk. Additional research is needed to determine whether genotype–phenotype correlations can serve as an additional means of risk stratification. Finally, our adequately powered study did not find evidence of a POE in *MSH6*-associated LS.

AUTHOR CONTRIBUTIONS

Conceptualization: Maartje Nielsen. Data curation: Anne-Sophie van der Werf-'t Lam, Mandy Villasmil, and Carli M. Tops. *Formal analysis*: Anne-Sophie van der Werf-'t Lam, Mar Rodriguez-Girondo, Mev Dominguez-Valentin, and Pal Møller. *Funding acquisition*: Maartje Nielsen. *Investigation*: Anne-Sophie van der Werf-'t Lam. *Methodology*: Mar Rodriguez-Girondo. *Project administration*: Anne-Sophie van der Werf-'t Lam and Maartje Nielsen. *Resources*: Maartje Nielsen. *Supervision*: Manon Suerink and Maartje Nielsen. *Visualization*: Anne-Sophie van der Werf-'t Lam, Manon Suerink, and Maartje Nielsen. *Writing—original draft*: Anne-Sophie van der Werf-'t Lam. *Writing—review and editing*: Anne-Sophie van der Werf-'t Lam, Mar Rodriguez-Girondo, Carli M. Tops, Liselotte van Hest, Hans J. P. Gille, Floor A. M. Duijkers, Anja Wagner, Ellis Eikenboom, Tom G. W. Letteboer, Mirjam M. de Jong, Sanne W. Bajwa-ten Broeke, Fonnet Bleeker, Encarna B. Gomez Garcia, Mev Dominguez-Valentin, Pal Møller, Manon Suerink, and Maartje Nielsen.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data from the Dutch cohort used during the current study are available from the corresponding author on reasonable request. Data from the PLSD cohort were provided by the PLSD with permission. Data will be shared upon reasonable request to the corresponding author with the permission of the PLSD.

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REFERENCES

1. Tamura K, Kaneda M, Futagawa M, et al. Genetic and genomic basis of the mismatch repair system involved in Lynch syndrome. *Int J Clin Oncol*. 2019;24(9):999-1011. doi:10.1007/s10147-019-01494-y
2. Dominguez-Valentin M, Sampson JR, Seppälä TT, et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the Prospective Lynch Syndrome Database. *Genet Med*. 2020;22(1):15-25. doi:10.1038/s41436-019-0596-9
3. Møller P, Seppälä 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*. 2018;67(7):1306-1316. doi:10.1136/gutjnl-2017-314057
4. Ten Broeke SW, van der Klift HM, Tops CMJ, et al. Cancer risks for PMS2-associated Lynch syndrome. *J Clin Oncol*. 2018;36(29):2961-2968. doi:10.1200/jco.2018.78.4777
5. Pérez-Cabornero L, Infante M, Velasco E, Lastra E, Miner C, Durán M. Genotype-phenotype correlation in MMR mutation-positive families with Lynch syndrome. *Int J Colorectal Dis*. 2013;28(9):1195-1201. doi:10.1007/s00384-013-1685-x
6. International Mismatch Repair Consortium. Variation in the risk of colorectal cancer in families with Lynch syndrome: a retrospective cohort study. *Lancet Oncol*. 2021;22(7):1014-1022. doi:10.1016/s1470-2045(21)00189-3
7. Peltomäki P, Gao X, Mecklin JP. Genotype and phenotype in hereditary nonpolyposis colon cancer: a study of families with different

vs. shared predisposing mutations. *Fam Cancer*. 2001;1(1):9-15. doi:10.1023/a:1011564720772
8. Geary J, Sasieni P, Houlston R, et al. Gene-related cancer spectrum in families with hereditary non-polyposis colorectal cancer (HNPCC). *Fam Cancer*. 2008;7(2):163-172. doi:10.1007/s10689-007-9164-6
9. Ryan NAJ, Morris J, Green K, et al. Association of mismatch repair mutation with age at cancer onset in Lynch syndrome: implications for stratified surveillance strategies. *JAMA Oncol*. 2017;3(12):1702-1706. doi:10.1001/jamaoncol.2017.0619
10. Gemechu SD, van Vliet CM, Win AK, et al. Do the risks of Lynch syndrome-related cancers depend on the parent of origin of the mutation? *Fam Cancer*. 2020;19(3):215-222. doi:10.1007/s10689-020-00167-4
11. van Vliet CM, Dowty JG, van Vliet JL, et al. Dependence of colorectal cancer risk on the parent-of-origin of mutations in DNA mismatch repair genes. *Hum Mutat*. 2011;32(2):207-212. doi:10.1002/humu.21408
12. Farrell MP, Hughes DJ, Schmid J, et al. Investigating parent of origin effects (POE) and anticipation in Irish Lynch syndrome kindreds. *J Clin Oncol*. 2013;31:431. doi:10.1200/jco.2013.31.4_suppl.431
13. Green J, O'Driscoll M, Barnes A, et al. Impact of gender and parent of origin on the phenotypic expression of hereditary nonpolyposis colorectal cancer in a large Newfoundland kindred with a common *MSH2* mutation. *Dis Colon Rectum*. 2002;45(9):1223-1232. doi:10.1007/s10350-004-6397-4
14. Suerink M, van der Klift HM, ten Broeke SW, et al. The effect of genotypes and parent of origin on cancer risk and age of cancer development in *PMS2* mutation carriers. *Genet Med*. 2016;18(4):405-409. doi:10.1038/gim.2015.83
15. 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*. 2004;96(4):261-268. doi:10.1093/jnci/djh034
16. Seppälä TT, Latchford A, Negoi I, et al. European guidelines from the EHTG and ESCP for Lynch syndrome: an updated third edition of the Mallorca guidelines based on gene and gender. *Br J Surg*. 2021;108(5):484-498. doi:10.1002/bjs.11902
17. van Puijenbroek M, Nielsen M, Reinards TH, et al. The natural history of a combined defect in *MSH6* and *MUTYH* in a HNPCC family. *Fam Cancer*. 2007;6(1):43-51. doi:10.1007/s10689-006-9103-y
18. van der Werf-'t Lam AS, Terlouw D, Tops CM, et al. Discordant staining patterns and microsatellite results in tumors of *MSH6* pathogenic variant carriers. *Mod Pathol*. 2023;36(9):100240. doi:10.1016/j.modpat.2023.100240
19. Dominguez-Valentin M, Haupt S, Seppälä TT, et al. Mortality by age, gene and gender in carriers of pathogenic mismatch repair gene variants receiving surveillance for early cancer diagnosis and treatment: a report from the prospective Lynch syndrome database. *EClinicalMedicine*. 2023;58:101909. doi:10.1016/j.eclinm.2023.101909
20. Inácio A, Silva AL, Pinto J, et al. Nonsense mutations in close proximity to the initiation codon fail to trigger full nonsense-mediated mRNA decay. *J Biol Chem*. 2004;279(31):32170-32180. doi:10.1074/jbc.M405024200
21. Shyu AB, Wilkinson MF, Van Hoof A. Messenger RNA regulation: to translate or to degrade. *EMBO J*. 2008;27(3):471-481. doi:10.1038/sj.emboj.7601977
22. Arntzen VH, Fiocco M, Lakeman IMM, Nielsen M, Rodríguez-Girondo M. A new inverse probability of selection weighted Cox model to deal with outcome-dependent sampling in survival analysis. *bioRxiv*. 2023. doi:10.1101/2023.02.07.527426
23. Baglietto L, Lindor NM, Dowty JG, et al. Risks of Lynch syndrome cancers for *MSH6* mutation carriers. *J Natl Cancer Inst*. 2010;102(3):193-201. doi:10.1093/jnci/djp473
24. di Masi A. May a missense mutation be more deleterious than a truncating mutation? *IUBMB Life*. 2008;60(1):79-81. doi:10.1002/iub.2
25. Kosho T, Okamoto N, Coffin-Siris Syndrome International Collaborators. Genotype-phenotype correlation of Coffin-Siris syndrome caused by mutations in *SMARCB1*, *SMARCA4*, *SMARCE1*, and *ARID1A*. *Am J Med Genet C Semin Med Genet*. 2014;166c:262-275. doi:10.1002/ajmg.c.31407
26. Sievänen T, Törmäkangas T, Laakkonen EK, et al. Body weight, physical activity, and risk of cancer in Lynch syndrome. *Cancers*. 2021;13(8):1849. doi:10.3390/cancers13081849
27. van Duijnhoven FJ, Botma A, Winkels R, Nagengast FM, Vasen HF, Kampman E. Do lifestyle factors influence colorectal cancer risk in Lynch syndrome? *Fam Cancer*. 2013;12(2):285-293. doi:10.1007/s10689-013-9645-8
28. Burn J, Sheth H, Elliott F, et al. Cancer prevention with aspirin in hereditary colorectal cancer (Lynch syndrome), 10-year follow-up and registry-based 20-year data in the CAPP2 study: a double-blind, randomised, placebo-controlled trial. *Lancet*. 2020;395(10240):1855-1863. doi:10.1016/s0140-6736(20)30366-4
29. Donald N, Malik S, McGuire JL, Monahan KJ. The association of low penetrance genetic risk modifiers with colorectal cancer in Lynch syndrome patients: a systematic review and meta-analysis. *Fam Cancer*. 2018;17(1):43-52. doi:10.1007/s10689-017-9995-8

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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