





















Article

No Difference in Penetrance between Truncating and Missense/Aberrant Splicing Pathogenic Variants in *MLH1* and *MSH2*: A Prospective Lynch Syndrome Database Study

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Abstract: Background. Lynch syndrome is the most common genetic predisposition for hereditary cancer. Carriers of pathogenic changes in mismatch repair (MMR) genes have an increased risk of developing colorectal (CRC), endometrial, ovarian, urinary tract, prostate, and other cancers, depending on which gene is malfunctioning. In Lynch syndrome, differences in cancer incidence (penetrance) according to the gene involved have led to the stratification of cancer surveillance. By contrast, any differences in penetrance determined by the type of pathogenic variant remain unknown. Objective. To determine cumulative incidences of cancer in carriers of truncating and missense or aberrant splicing pathogenic variants of the *MLH1* and *MSH2* genes. Methods. Carriers of pathogenic variants of *MLH1* (*path_MLH1*) and *MSH2* (*path_MSH2*) genes filed in the Prospective Lynch Syndrome Database (PLSD) were categorized as truncating or missense/aberrant splicing

according to the InSiGHT criteria for pathogenicity. Results. Among 5199 carriers, 1045 had missense or aberrant splicing variants, and 3930 had truncating variants. Prospective observation years for the two groups were 8205 and 34,141 years, respectively, after which there were no significant differences in incidences for cancer overall or for colorectal cancer or endometrial cancers separately. Conclusion. Truncating and missense or aberrant splicing pathogenic variants were associated with similar average cumulative incidences of cancer in carriers of *path MLH1* and *path_MSH2*.

Keywords: *MLH1*; *MSH2*; penetrance; cancer incidence; truncating; missense; aberrant splicing; Lynch syndrome

1. Introduction

Lynch syndrome (LS) is a common, dominantly inherited cancer syndrome caused by pathogenic variants of mismatch repair genes (*path_MMR*) [1–4] and affects an estimated 1 in 300 individuals. *Path_MMR* carriers have increased incidences of cancers of the colon, rectum (often grouped as colorectal cancer, CRC), endometrium, ovaries, stomach, small bowel, bile duct, pancreas, and upper urinary tract [1,4–6]. The cancers may occur much earlier in life than their sporadic counterparts, and penetrance and expression vary by gene and by gender from very high to not measurable [7]. Factors considered likely to contribute to both incomplete penetrance and variation in cancer incidence in different organs include environmental factors, modifying genetic factors, and the nature of the pathogenic variants themselves. Genetic association studies have examined the relationship between variants elsewhere in the genome and cancer incidence in LS individuals and have suggested that SNPs at 8q23.3 (rs16892766) and 11q23.1 (rs3802842) are associated with increased LS CRC risk, especially for female *MLH1* carriers [8,9]. By contrast, a recent study did not find any risk-modifying effects of these SNPs in a cohort of 507 *PMS2* carriers [10]. Additional factors implicated in phenotypic variability in LS include epigenetic regulators, microRNAs, hormonal factors, acetyl-salicylic acid prophylaxis, smoking, and body mass index. In the current study, which addresses the question of whether penetrance varies according to the type of *path_MMR* variant, such modifying factors are not expected to be stratified by the type of *path_MMR* variant.

According to the InSiGHT database (<https://www.insight-group.org/variants/databases/>, accessed on 12 February 2021), more than 3000 different pathogenic or likely pathogenic (class 5 or 4 and, therefore, clinically actionable) germline sequence variants have been deposited for the MMR genes, of which 40% have been identified in *MLH1*, 34% in *MSH2*, 18% in *MSH6*, and 8% in *PMS2* [11,12]. Approximately 50% of those in *MLH1* are missense variants [13–16], whereas most affecting *MSH2* are nonsense, frameshift, or splice site changes, which can be considered *a priori* to be pathogenic [13–16]. Pathogenic variants that result in aberrant splicing may be associated with lower penetrance compared to truncating variants of the same gene [17]. Recent studies have shown that some exonic missense variants (and some synonymous variants) cause disease through interference with the splicing machinery, adding complexity to the classification of variants [18–20]. The potential for clinically relevant associations with different types of germline variants in LS was illustrated by a recent study that reported a significantly better prognosis for CRC in LS patients who had missense or splice site *path_MMR* variants compared to those with frameshift or nonsense variants or large genomic rearrangements (overall survival 132.5 vs. 82.5 months) [21]. In contrast, one retrospective study suggested an increased risk for endometrial cancer in carriers of missense *path_MLH1* variants, but this was not seen in other cancers [6].

We here report prospectively observed, cumulative incidences of cancer in *path_MLH1* and *path_MSH2* carriers with truncating versus predicted missense and non-canonical aberrant splicing pathogenic variants to explore the hypothesis that carriers of truncating variants have higher cancer incidence.

2. Methods

2.1. The Prospective Lynch Syndrome Database (PLSD) Design

We analysed carriers of *path_MLH1* and *path_MSH2* variants from the PLSD. The PLSD design and its inclusion criteria have been described previously in detail [1–4,7]. In brief, the PLSD is an international prospective observational study including centres from 18 countries worldwide. Data were collected from the first prospectively planned and completed colonoscopy onwards, and all recruits had subsequent follow-up of one year or more. A detailed discussion of methods is given in Moller et al. and Seppälä et al. [7,22]. Time to first cancer after inclusion was calculated for each organ or group of organs. When calculating the time to any cancer (penetrance), only patients without any cancer prior to or at inclusion were counted. For each calculation, each patient was censored at the first event or last observation, whichever came first. The number of observation years and cancers in the 5-year groups were counted from 25 to 75 years and the corresponding annual cancer incidence rates by age group were calculated.

2.2. MMR Gene Variant Categorization

Path_MLH1 and *path_MSH2* variants that were classified as clinically actionable (class 4 and 5) in the InSIGHT database [23] were grouped as: (1) truncating (including frameshift, nonsense, deletion of exon(s), and canonical splicing); (2) missense/aberrant splicing (aberrant splicing determined by splicing assay of intronic variants outside the canonical +/− 2(3) positions or exonic variants), and (3) others (including in-frame deletions or duplications, duplications of whole exons, initiation codon variants, intronic variants, and variants not compliant with any of the categories described). The groups of truncating and missense/aberrant splicing variants were used for calculations. As previously reported [1], the number of carriers with *path_MSH6* or *path_PMS2* variants were limited and considered insufficient for the analyses presented in this report.

2.3. Cancer Risk by Gene and Type of Genetic Variant

The cumulative incidence (Q) and the annual incidence rates (AIRs) by age were calculated as previously described [1]. In brief, Q was computed starting at age 25, assuming zero incidence rate before age 25, using the formula $Q(\text{age}) = Q(\text{age} - 1) + (1 - Q(\text{age} - 1)) \times \text{AIR}(\text{age})$, where $\text{AIR}(\text{age})$ is the annual incidence rate as estimated from the corresponding 5-year interval. Confidence intervals were calculated as previously described [1].

2.4. Ethics Statement

All reporting centers exported de-identified data to the PLSD, and the patients had been followed up prospectively according to local clinical guidelines, as previously described [1–4,24,25].

3. Results

3.1. Characterization of *Path_MLH1* and *Path_MSH2* Genetic Variants

Numbers of carriers and follow-up times by gene, variant type—missense/aberrant splicing or truncating or other—are detailed in Table 1. In sum, 1045 carriers with missense/aberrant splicing variants were followed for an average of 7.9 years (95% CI (7.6–8.2)), 3930 carriers with truncating variants were followed for an average of 8.7 years (95% CI (8.5–8.9)), and 224 carriers had other types of variants (Table 1). Because carriers of *path_MLH1* and *path_MSH2* have different incidences of cancers [1], the incidences in this report were calculated for each gene separately.

Table 1. Categorization of the *path_MLH1* and *path_MSH2* carriers having inherited variants causing truncating or missense/aberrant splicing clinically actionable variants as defined in the InSiGHT database.

Categorization Group	Variant Type	Gene	Number of Carriers	Sum of the FUP Years	Mean of the FUP Years	95% CI
Missense or aberrant splicing	Aberrant Splicing	<i>MLH1</i>	233	1829	7.8	(7.1–8.5)
	Aberrant Splicing	<i>MSH2</i>	350	2778	7.9	(7.4–8.4)
	Missense	<i>MLH1</i>	345	2715	7.9	(7.4–8.4)
	Missense	<i>MSH2</i>	117	883	7.5	(6.7–8.3)
	Total		1045	8205	7.9	(7.6–8.2)
Truncating	Canonical Splicing	<i>MLH1</i>	501	4709	9.4	(8.9–9.9)
	Canonical Splicing	<i>MSH2</i>	185	1635	8.8	(8.0–9.6)
	Exon Deletion	<i>MLH1</i>	688	7643	11.1	(10.6–11.6)
	Exon Deletion	<i>MSH2</i>	579	4207	7.3	(6.9–7.7)
	Nonsense	<i>MLH1</i>	324	2880	8.9	(8.3–9.5)
	Nonsense	<i>MSH2</i>	608	4929	8.1	(7.7–8.5)
	Frameshift	<i>MLH1</i>	482	3722	7.7	(7.3–8.1)
	Frameshift	<i>MSH2</i>	563	4416	7.8	(7.4–8.2)
	Total		3930	34,141	8.7	(8.5–8.9)
Others	Exon Duplication	<i>MLH1</i>	1	1	1	(1.0–1.0)
	Exon Duplication	<i>MSH2</i>	16	71	4.4	(2.7–6.1)
	Inframe Indel	<i>MLH1</i>	85	790	9.3	(8.3–10.3)
	Inframe Indel	<i>MSH2</i>	93	811	8.7	(7.7–9.7)
	Initiation Codon	<i>MLH1</i>	8	36	4.5	(1.5–7.5)
	Intronic	<i>MSH2</i>	3	25	8.3	(2.1–14.5)
	Undefined	<i>MLH1</i>	18	249	13.8	(10.7–16.9)
	Total		224	1983		

FUP, follow-up years.

For the *MLH1* gene, missense variants were more frequent (60.7%, 345/578) than aberrant splicing variants (40.3%, 233/578), while for the *MSH2* gene, aberrant splicing variants were more common than missense variants (75%, 350/467 vs. 26%, 117/467) ($p > 0.05$). Truncating variants affected both genes in an equal proportion (50% each). Within the set of truncating variants ($n = 3930$), the most common types with respect to variant consequence were exon or multi-exon deletions (32%, 1267/3930), followed by frameshift (27%, 1045/3930) and nonsense (24%, 932/3930). By the type of truncating variant and gene, exon or multi-exon deletions were the more frequent variant in *MLH1* (34.5%, 688/1995), followed by canonical splicing variants (25.1%, 501/1995), frameshift (24.1%, 482/1995), and nonsense variants (16.2%, 324/1995), while for the *MSH2* gene, nonsense variants were the most frequent (31.4%, 608/1935) ($p > 0.05$) (Table 1).

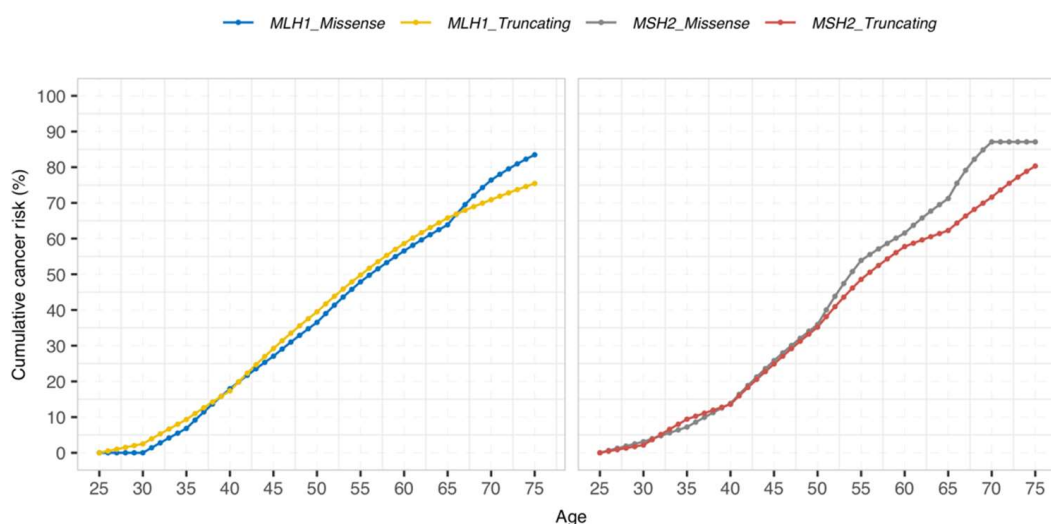
3.2. Cumulative Cancer Incidence by Gene and Type of Genetic Variant

The cumulative incidences by gene for any cancer, CRC, and endometrial cancer are detailed in Table 2 and illustrated in Figure 1. There were no significant differences between carriers with missense/aberrant splicing versus truncating variants at any age in any group. Moreover, no differences which could be considered non-significant trends were observed ($p > 0.05$ for all comparisons).

Table 2. Cumulative cancer incidences stratified by age, gene, variant, and organ.

		Cumulative Incidences (95% CI)			
	Age	<i>MLH1</i> Missense/Aberrant Splicing	<i>MLH1</i> Truncating	<i>MSH2</i> Missense/Aberrant Splicing	<i>MSH2</i> Truncating
Any cancer	30	0 (0–0)	2.5 (0.3–4.7)	3.1 (0–9.1)	2.2 (0–4.6)
	40	17.9 (9.3–26.5)	17.3 (13.1–21.6)	13.8 (4.1–23.5)	13.6 (8.9–18.2)
	50	36.5 (26.6–46.5)	39.5 (34.5–44.5)	36.0 (23.9–48.0)	35.2 (29.3–41.1)
	60	56.6 (44.6–68.4)	58.6 (53.4–63.9)	61.6 (49.5–73.6)	57.8 (51.4–64.1)
	70	76.4 (63.6–89.2)	71.0 (65.1–76.7)	87.1 (75.6–98.6)	71.6 (64.4–78.8)
	75	83.5 (71.4–95.6)	75.4 (69.1–81.8)	87.1 (75.6–98.6)	80.3 (73.3–87.4)
Colorectal cancer	30	0 (0–0)	2.5 (0.3–4.6)	0 (0–0)	2.1 (0–4.4)
	40	14.5 (6.5–22.6)	14.6 (10.6–18.6)	7.0 (0.3–13.6)	8.3 (4.5–12.1)
	50	23.8 (14.6–33.0)	28.0 (23.3–32.7)	15.1 (6.4–23.8)	18.1 (13.3–22.9)
	60	38.4 (26.5–50.4)	38.9 (33.7–44.0)	30.6 (19.7–41.5)	28.9 (23.3–34.5)
	70	53.7 (39.0–68.3)	47.0 (41.2–52.8)	49.9 (36.4–63.4)	41.1 (34.2–48.0)
	75	61.6 (45.9–77.4)	50.3 (43.8–56.8)	49.9 (36.4–63.4)	47.3 (39.6–55.1)
Endometrial cancer	30	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	40	5.2 (0–10.9)	0.5 (0–1.5)	2.5 (0–7.2)	2.4 (0–5.0)
	50	11.8 (3.5–20.0)	15.0 (10.1–19.9)	13.3 (2.4–24.2)	19.5 (12.9–26.1)
	60	27.0 (13.9–40.1)	27.7 (21.0–34.3)	34.5 (17.6–51.3)	39.2 (30.3–48.1)
	70	34.9 (19.2–50.6)	35.9 (27.6–44.2)	45.6 (25.6–65.6)	48.0 (37.4–58.5)
	75	34.9 (19.2–50.6)	38.2 (29.0–47.4)	45.6 (25.6–65.6)	50.9 (39.5–62.3)

CI, confidence interval.



(a)

Figure 1. Cont.

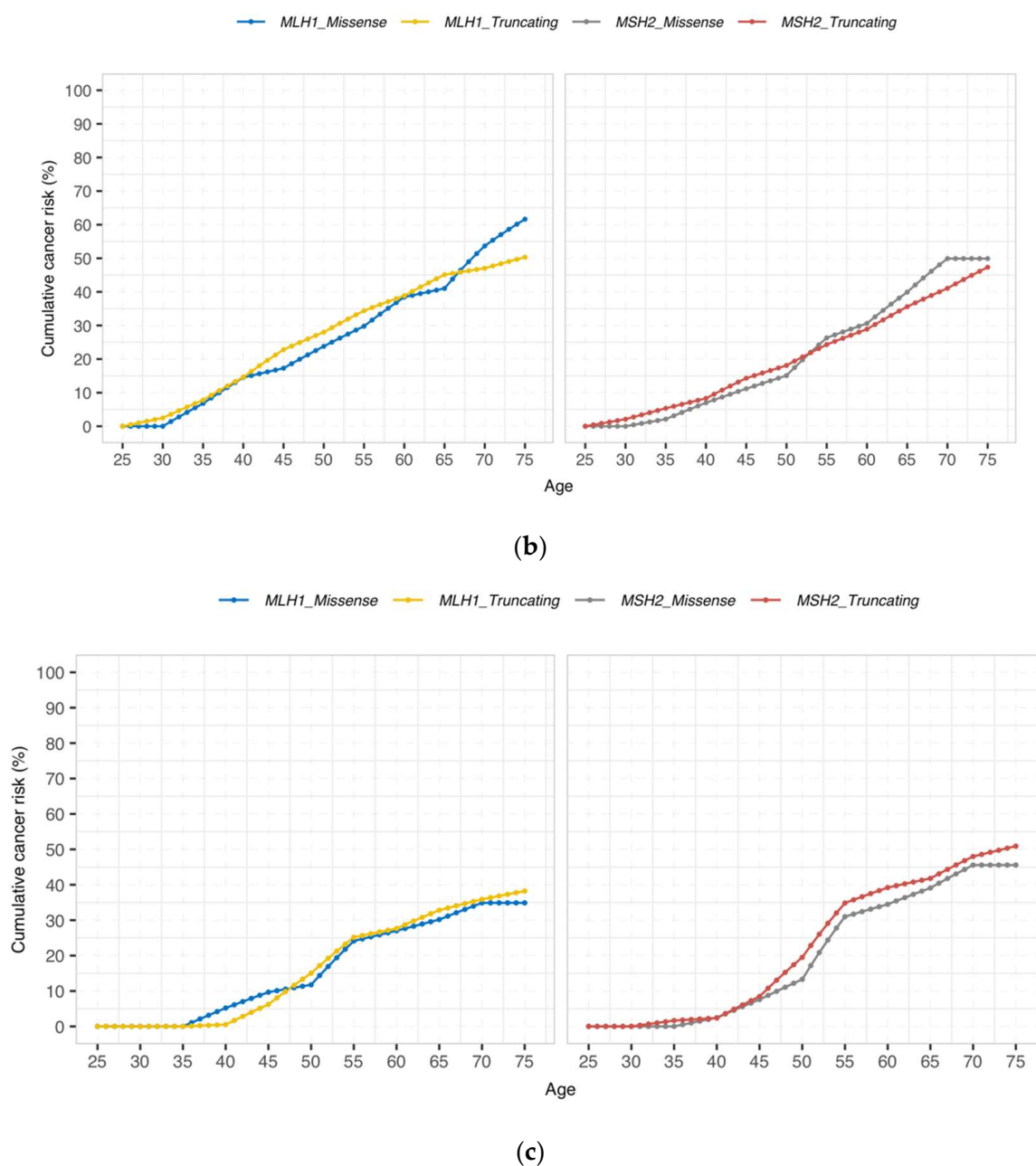


Figure 1. Cumulative incidence of (a) any cancer, (b) colorectal, and (c) endometrial cancer by gene and type of variant. There were no significant differences between carriers with missense/aberrant splicing versus truncating variants at any age in any groups ($p > 0.05$ for all comparisons).

Cumulative incidences for any cancer at 50 years in *path_MLH1* carriers with truncating or missense/aberrant splicing variants were 39.5% (95% CI (34.5–44.5)) and 36.5% (95% CI (26.6–46.5)), respectively, and in *path_MSH2* carriers, 35.2% (95% CI (29.3–41.1)) and 36.0% (95% CI (23.9–48.0)), respectively. Corresponding cumulative incidences for CRC were 28.0% (95% CI (23.3–32.7)) versus 23.8% (95% CI (14.6–33.0)) for *path_MLH1* carriers and 18.1% (95% CI (13.3–22.9)) versus 15.1% (95% CI (6.4–23.8)) for *path_MSH2* carriers with truncating or missense/aberrant splicing variants, respectively. Corresponding cumulative incidences for endometrial cancer were 15.0% (95% CI (10.1–19.9)) versus 11.8% (95% CI (3.5–20.0)) for *path_MLH1* carriers and 19.5% (95% CI (12.9–26.1)) versus 13.3% (95% CI (2.4–24.2)) for *path_MSH2* carriers with truncating or missense/aberrant splicing variants, respectively.

Cumulative incidences for any cancer at 75 years in *path_MLH1* carriers with truncating or missense/aberrant splicing variants were 75.4% (95% CI (69.1–81.8)) versus 83.5% (95% CI (71.4–95.6)), respectively, and in *path_MSH2* carriers 80.3% (95% CI (73.3–87.4)) versus 87.1% (95% CI (75.6–98.6)), respectively. Corresponding cumulative incidences for CRC were 50.3% (95% CI (43.8–56.8)) versus 61.6% (95% CI (45.9–77.4)) for *path_MLH1* carriers and 47.3% (95% CI (39.6–55.1)) versus 49.9% (95% CI (36.4–63.4)) for *path_MSH2* carriers with truncating or missense/aberrant splicing variants, respectively. Corresponding cumulative incidences for endometrial cancer were 38.2% (95% CI (29.0–47.4)) versus 34.9% (95% CI (19.2–50.6)) for *path_MLH1* carriers and 50.9% (95% CI (39.5–62.3)) versus 45.6% (95% CI (25.6–65.6)) for *path_MSH2* carriers with truncating or missense/aberrant splicing variants, respectively.

4. Discussion and Conclusions

In contrast to expectations for the hypothesis we tested, carriers of truncating variants of either *path_MLH1* and *path_MSH2* had similar average cumulative incidences of cancers to carriers of missense or aberrant splicing variants affecting the corresponding gene. On average, carriers of both categories of pathogenic variants had the same high cumulative incidences of any cancer for both genes. The numbers of carriers in each of the groups were large enough to detect any major differences. Our findings will be of clinical interest when interpreting the results of genetic testing, and in planning preventive health care interventions in carriers. As reported previously [1,4], *path_MSH2* carriers have higher incidence of other cancers than in the colorectum and endometrium, which is also reflected in the current results for carriers of both truncating and missense *path_MSH2* variants. The cumulative cancer incidences for missense *path_MSH2* carriers in the two highest age groups showed variation that was considered likely to be stochastic, reflecting the limited number of observation years.

We have previously reported that pathogenic variants in each of the MMR genes result in different risks for cancers in organs, including the colorectum, endometrium, ovaries, stomach, small bowel, bile duct, pancreas, and upper urinary tract [1]. Previously, only one study with a very limited number of cases attempted to address the issue of whether the type of pathogenic variant also resulted in different cancer risks but was inconclusive [13].

Some MMR gene variants may be associated with partial but compromised function. The POLYPHEN and SIFT algorithms [26,27] attribute distinct degrees of malfunctioning to different missense variants, and there are examples of aberrant splicing and missense variants in the *BRCA*-genes that are associated with intermediate cancer incidences [22,28,29]. We cannot rule out the possibility that the criteria applied by the InSiGHT database to classify variants lack the sensitivity to identify low-risk variants in *MLH1* and *MSH2*. Indeed, a functional study showed that the *MLH1* variant p.K618T that was classified benign by InSiGHT had an intermediate repair capacity of ~35% to 50% [30]. Carriers of such variants may be at moderately increased risk for cancer, but may not be offered appropriate health care. Less penetrant *path_MMR* variants may also present clinically as the autosomal recessive constitutional mismatch repair deficiency syndrome (CCMRD), but *path_PMS2* and *path_MSH6* variants account for the majority of such cases. *Path_PMS2* variants associated with a milder heterozygous phenotype may be overrepresented, since it was shown that heterozygous relatives of CMMRD patients had a lower cumulative colon cancer risk (8.7%) than reported for *path_PMS2* as a whole by the PLSD and others [1,31]. A difference in age at CRC diagnosis was found for *path_PMS2* carriers when stratifying variants into those that lead to loss of RNA expression compared to those for which expression was preserved [32,33], but a similar relationship was not observed in a CMMRD family cohort. Host immune factors may also be involved in determining cancer incidence in LS. Carriers of *path_MLH1* and *path_MSH2* variants develop thousands of mismatch repair-deficient and potentially precancerous gastrointestinal crypts [34,35]. The frequency at which they progress to infiltrating cancers may be largely determined by the host immune system, rather than the nature of the inherited *path_MMR* variant [34,36]. Genetic modifiers may

also contribute to the variation in cancer risk and phenotypic variability in *path_MMR* carriers, leading those with such genetic modifiers to be at increased risk of having further cancers [9].

The strengths of our study include its large sample size and its prospective design, but a potential weakness is selection bias at contributing centres that may have failed to identify some low-penetrance variants. We are also aware that there are many other possible categorizations of *path_MMR* variants that could be investigated for differences in associated cancer incidences using the PLSD data, but we hesitate to do so until we have other plausible hypotheses to test. Similarly, we did not test for differences between class 4 and class 5 variants in relation to cancer incidence, as numbers were not large enough to make this comparison.

The penetrance of the pathogenic MMR variants has no bearing on the classification of their pathogenicity. Having recruited sufficient numbers of carriers into PLSD to reach robust conclusions, we examined the hypothesis that missense or aberrant splicing variants may have lower incidence of cancer than truncating *path_MLH1* and *path_MSH2* variants. In contrast to our hypothesis, we found no difference. The results are of practical interest when presenting preventive health care options to carriers of *path_MLH1* and *path_MSH2* variants.

Author Contributions: P.M., J.-P.P. and M.D.-V. designed the study. M.D.-V. is the PLSD curator, J.-P.P. is the InSiGHT database curator, and P.M. is the PI to the PLSD. J.-P.P. scored the variants as truncating or missense/aberrant splicing. S.N. implemented code for cumulative risk and performed Figure 1. M.D.-V. and P.M. calculated the results. M.D.-V., J.-P.P., J.R.S., T.T.S. and P.M. wrote the manuscript. All authors contributed data to the PLSD and reviewed and approved the manuscript. All authors have read and agreed to the final version of the manuscript.

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Institutional Review Board Statement: The study adhered to the principles set out in the Declaration of Helsinki. It was approved by the Oslo University Hospital ethical committee approval code S-02030 and its data governance rules by the Norwegian Data Inspectorate ref. 2001/2988-2. Genetic testing was performed with informed consent according to local and national requirements and all reporting centers exported only de-identified data to PLSD.

Informed Consent Statement: Not applicable.

Data Availability Statement: The cancer risk algorithm is available at the PLSD website (www.plsd.eu, accessed on 12 February 2021) that is based upon the results presented in this report and enables interactive calculation of remaining lifetime risks for cancer in any LS patient by giving their age, gender, and gene variant.

Conflicts of Interest: Seven authors (S.A., C.P., R.H., E.H.-F., V.S.-L., G.C., J.B.) of this publication are members of the European Reference Network on Genetic Tumor Risk Syndromes (ERN GENTURIS)-Project ID No. 739547. R.B. has received honoraria for lectures and advisory boards from AbbVie, Amgen, AstraZeneca, Bayer, BMS, Boehringer-Ingelheim, Illumina, Lilly, Merck-Serono, MSD, Novartis, Qiagen, Pfizer, Roche, and Targos MP Inc. R.B. is a Co-Founder and Scientific Advisor for Targos Mol. Pathology Inc. Kassel/Germany. T.T.S. is the CEO and co-owner of Healthfund Finland Oy and reports an interview honorarium from Boehringer Ingelheim Finland. J.B. has received honoraria for advisory boards from Astra Zeneca and MSD. G.C. is founder and co-owner of VCN Biosciences, Spain.

References

1. Dominguez-Valentin, M.; Sampson, J.R.; Seppälä, T.T.; Broeke, S.W.T.; Be, J.-P.P.; Nakken, S.; Engel, C.; Aretz, S.; Jenkins, M.; Sunde, L.; 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*, 15–25. [[CrossRef](#)]
2. Møller, P.; Seppälä, T.; Bernstein, I.; Holinski-Feder, E.; Sala, P.; Evans, D.G.; Lindblom, A.; Macrae, F.; Blanco, I.; Sijmons, R.; et al. Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: First report from the prospective Lynch syndrome database. *Gut* **2017**, *66*, 464–472. [[CrossRef](#)]
3. Møller, P.; Seppälä, T.; Bernstein, I.; Holinski-Feder, E.; Sala, P.; Evans, D.G.; Lindblom, A.; Macrae, F.; Blanco, I.; Sijmons, R.; et al. Incidence of and survival after subsequent cancers in carriers of pathogenic MMR variants with previous cancer: A report from the prospective Lynch syndrome database. *Gut* **2017**, *66*, 1657–1664. [[CrossRef](#)]
4. Møller, P.; Seppälä, T.; Bernstein, I.; Holinski-Feder, E.; Sala, P.; Evans, D.G.; Lindblom, A.; Macrae, F.; Blanco, I.; Sijmons, R.H.; 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*, 1306–1316. [[CrossRef](#)]
5. Lynch, H.T.; Snyder, C.L.; Shaw, T.G.; Heinen, C.D.; Hitchins, M.P. Milestones of Lynch syndrome: 1895–2015. *Nat. Rev. Cancer* **2015**, *15*, 181–194. [[CrossRef](#)] [[PubMed](#)]
6. Ryan, N.; Morris, J.; Green, K.; Laloo, F.; Woodward, E.R.; Hill, J.; Crosbie, E.J.; Evans, D.G. Association of Mismatch Repair Mutation with Age at Cancer Onset in Lynch Syndrome. *JAMA Oncol.* **2017**, *3*, 1702–1706. [[CrossRef](#)]
7. Seppälä, T.T.; Dominguez-Valentin, M.; Sampson, J.R.; Møller, P. Prospective observational data informs understanding and future management of Lynch syndrome: Insights from the Prospective Lynch Syndrome Database (PLSD). *Fam. Cancer* **2021**, *20*, 35–39. [[CrossRef](#)] [[PubMed](#)]
8. Wijnen, J.T.; Brohet, R.M.; van Eijk, R.; Jagmohan-Changur, S.; Middeldorp, A.; Tops, C.M.; Van Puijenbroek, M.; Ausems, M.G.E.M.; García, E.G.; Hes, F.J.; et al. Chromosome 8q23.3 and 11q23.1 Variants Modify Colorectal Cancer Risk in Lynch Syndrome. *Gastroenterology* **2009**, *136*, 131–137. [[CrossRef](#)]
9. Talseth-Palmer, B.A.; Wijnen, J.T.; Brenne, I.S.; Jagmohan-Changur, S.; Barker, D.; Ashton, K.A.; Tops, C.M.; Evans, T.-J.; McPhillips, M.; Groombridge, C.; 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* **2012**, *132*, 1556–1564. [[CrossRef](#)] [[PubMed](#)]
10. Ten Broeke, S.W.; Van Der Klift, H.M.; Tops, C.M.; Aretz, S.; Bernstein, I.; Buchanan, D.D.; De La Chapelle, A.; Capella, G.; Clendenning, M.; Engel, C.; et al. Cancer Risks for PMS2-Associated Lynch Syndrome. *J. Clin. Oncol.* **2018**, *36*, 2961–2968. [[CrossRef](#)]
11. Peltomäki, P.; Olkinuora, A.; Nieminen, T.T. Updates in the field of hereditary nonpolyposis colorectal cancer. *Expert Rev. Gastroenterol. Hepatol.* **2020**, *14*, 707–720. [[CrossRef](#)]
12. Peltomäki, P. Update on Lynch syndrome genomics. *Fam. Cancer* **2016**, *15*, 385–393. [[CrossRef](#)]
13. Cravo, M.; Afonso, A.; Lage, P.; Albuquerque, C.; Maia, L.; Lacerda, C.; Fidalgo, P.; Chaves, P.; Cruz, C.; Nobre-Leitão, C. Pathogenicity of missense and splice site mutations in hMSH2 and hMLH1 mismatch repair genes: Implications for genetic testing. *Gut* **2002**, *50*, 405–412. [[CrossRef](#)]
14. Liu, B.; Parsons, R.E.; Hamilton, S.R.; Petersen, G.M.; Lynch, H.T.; Watson, P.; Markowitz, S.; Willson, J.K.V.; Green, J.; de la Chapelle, A.; et al. hMSH2 mutations in hereditary nonpolyposis colorectal cancer kindreds. *Cancer Res.* **1994**, *54*, 4590–4594. [[PubMed](#)]
15. Papadopoulos, N.; Nicolaidis, N.C.; Wei, Y.F.; Ruben, S.M.; Carter, K.C.; Rosen, C.A.; Haseltine, W.A.; Fleischmann, R.D.; Fraser, C.; Adams, M.D.; et al. Mutation of a mutL homolog in hereditary colon cancer. *Science* **1994**, *263*, 1625–1629. [[CrossRef](#)] [[PubMed](#)]
16. Peltomäki, P.; Vasen, H. Mutations predisposing to hereditary nonpolyposis colorectal cancer: Database and results of a collaborative study. The International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer. *Gastroenterology* **1997**, *113*, 1146–1158. [[CrossRef](#)]
17. Li, L.; Hamel, N.; Baker, K.; McGuffin, M.J.; Couillard, M.; Gologan, A.; Marcus, V.A.; Chodirker, B.; Chudley, A.; Stefanovici, C.; et al. A homozygous PMS2 founder mutation with an attenuated constitutional mismatch repair deficiency phenotype. *J. Med. Genet.* **2015**, *52*, 348–352. [[CrossRef](#)] [[PubMed](#)]
18. Auclair, J.; Busine, M.P.; Navarro, C.; Ruano, E.; Montmain, G.; Desseigne, F.; Saurin, J.C.; Lasset, C.; Bonadona, V.; Giraud, S.; et al. Systematic mRNA analysis for the effect of MLH1 and MSH2 missense and silent mutations on aberrant splicing. *Hum. Mutat.* **2006**, *27*, 145–154. [[CrossRef](#)]
19. Soukariéh, O.; Gaildrat, P.; Hamieh, M.; Drouet, A.; Baert-Desurmont, S.; Frébourg, T.; Tosi, M.; Martins, A. Exonic Splicing Mutations Are More Prevalent than Currently Estimated and Can Be Predicted by Using In Silico Tools. *PLoS Genet.* **2016**, *12*, e1005756. [[CrossRef](#)]
20. Thompson, B.A.; Walters, R.; Parsons, M.T.; Dumenil, T.; Drost, M.; Tiersma, Y.; Lindor, N.M.; Tavtigian, S.V.; De Wind, N.; Spurdle, A.B.; et al. Contribution of mRNA Splicing to Mismatch Repair Gene Sequence Variant Interpretation. *Front. Genet.* **2020**, *11*, 798. [[CrossRef](#)]
21. Maccaroni, E.; Bracci, R.; Giampieri, R.; Bianchi, F.; Belvederesi, L.; Brugiati, C.; Pagliaretta, S.; Del Prete, M.; Scartozzi, M.; Cascinu, S. Prognostic impact of mismatch repair genes germline defects in colorectal cancer patients: Are all mutations equal? *Oncotarget* **2015**, *6*, 38737–38748. [[CrossRef](#)]

22. Møller, P. The Prospective Lynch Syndrome Database reports enable evidence-based personal precision health care. *Hered. Cancer Clin. Pract.* **2020**, *18*, 6–7. [[CrossRef](#)]
23. Thompson, B.A.; Spurdle, A.B.; Plazzer, J.-P.; Greenblatt, M.S.; Akagi, K.; Al-Mulla, F.; Bapat, B.; Bernstein, I.; Capellá, G.; den Dunnen, J.T.; et al. Application of a 5-tiered scheme for standardized classification of 2360 unique mismatch repair gene variants in the InSiGHT locus-specific database. *Nat. Genet.* **2014**, *46*, 107–115. [[CrossRef](#)] [[PubMed](#)]
24. Seppälä, T.; Pylvänäinen, K.; Evans, D.G.; Järvinen, H.; Renkonen-Sinisalo, L.; Bernstein, I.; Holinski-Feder, E.; Sala, P.; Lindblom, A.; Macrae, F.; et al. Colorectal cancer incidence in path_MLH1 carriers subjected to different follow-up protocols: A Prospective Lynch Syndrome Database report. *Hered. Cancer Clin. Pract.* **2017**, *15*, 18. [[CrossRef](#)]
25. Seppälä, T.T.; Ahadova, A.; Dominguez-Valentin, M.; Macrae, F.; Evans, D.G.; Therkildsen, C.; Sampson, J.; Scott, R.; Burn, J.; Möslein, G.; et al. Lack of association between screening interval and cancer stage in Lynch syndrome may be accounted for by over-diagnosis; a prospective Lynch syndrome database report. *Hered. Cancer Clin. Pract.* **2019**, *17*, 8. [[CrossRef](#)]
26. Adzhubei, I.; Jordan, D.; Sunyaev, S.R. Predicting Functional Effect of Human Missense Mutations Using PolyPhen-2. *Curr. Protoc. Hum. Genet.* **2013**, *76*, 7.20.1–7.20.41. [[CrossRef](#)]
27. Ng, P.C.; Henikoff, S. SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res.* **2003**, *31*, 3812–3814. [[CrossRef](#)] [[PubMed](#)]
28. Moghadasi, S.; Meeks, H.D.; Vreeswijk, M.P.; Janssen, L.A.; Borg, Å.; Ehrencrona, H.; Paulsson-Karlsson, Y.; Wappenschmidt, B.; Engel, C.; Gehrig, A.; et al. The BRCA1 c. 5096G>A p.Arg1699Gln (R1699Q) intermediate risk variant: Breast and ovarian cancer risk estimation and recommendations for clinical management from the ENIGMA consortium. *J. Med. Genet.* **2017**, *55*, 15–20. [[CrossRef](#)]
29. Spurdle, A.B.; Whiley, P.J.; Thompson, B.; Feng, B.; Healey, S.; Brown, M.; Pettigrew, C.; Van Asperen, C.J.; Ausems, M.G.E.M.; Kattentidt-Mouravieva, A.A.; et al. BRCA1 R1699Q variant displaying ambiguous functional abrogation confers intermediate breast and ovarian cancer risk. *J. Med. Genet.* **2012**, *49*, 525–532. [[CrossRef](#)] [[PubMed](#)]
30. Drost, M.; Ms, Y.T.; Thompson, B.A.; Frederiksen, J.H.; Keijzers, G.; Glubb, D.; Kathe, S.; Osinga, J.; Westers, H.; Pappas, L.; et al. A functional assay-based procedure to classify mismatch repair gene variants in Lynch syndrome. *Genet. Med.* **2019**, *21*, 1486–1496. [[CrossRef](#)]
31. Ten Broeke, S.W.; Brohet, R.M.; Tops, C.M.; van der Klift, H.M.; Velthuisen, M.E.; Bernstein, I.; Munar, G.C.; Garcia, E.G.; Hoogerbrugge, N.; Letteboer, T.G.W.; et al. Lynch syndrome caused by germline PMS2 mutations: Delineating the cancer risk. *J. Clin. Oncol.* **2015**, *33*, 319–325. [[CrossRef](#)] [[PubMed](#)]
32. Suerink, M.; Rodríguez-Gironde, M.; Van Der Klift, H.M.; Colas, C.; Brugieres, L.; Lavoine, N.; Jongmans, M.; Munar, G.C.; Evans, D.G.; Farrell, M.P.; et al. An alternative approach to establishing unbiased colorectal cancer risk estimation in Lynch syndrome. *Genet. Med.* **2019**, *21*, 2706–2712. [[CrossRef](#)] [[PubMed](#)]
33. Suerink, M.; Van Der Klift, H.M.; Broeke, S.W.T.; Dekkers, O.M.; Bernstein, I.; Munar, G.C.; Garcia, E.G.; Hoogerbrugge, N.; Letteboer, T.G.W.; Menko, F.H.; et al. The effect of genotypes and parent of origin on cancer risk and age of cancer development in PMS2 mutation carriers. *Genet. Med.* **2015**, *18*, 405–409. [[CrossRef](#)]
34. Ballhausen, A.; Przybilla, M.J.; Jendrusch, M.; Haupt, S.; Pfaffendorf, E.; Seidler, F.; Witt, J.; Sanchez, A.H.; Urban, K.; Draxlbauer, M.; et al. The shared frameshift mutation landscape of microsatellite-unstable cancers suggests immunoeediting during tumor evolution. *Nat. Commun.* **2020**, *11*, 1–13. [[CrossRef](#)] [[PubMed](#)]
35. Ahadova, A.; Doeberitz, M.V.K.; Bläker, H.; Kloor, M. CTNNB1-mutant colorectal carcinomas with immediate invasive growth: A model of interval cancers in Lynch syndrome. *Fam. Cancer* **2016**, *15*, 579–586. [[CrossRef](#)] [[PubMed](#)]
36. Ahadova, A.; Seppälä, T.; Engel, C.; Gallon, R.; Burn, J.; Holinski-Feder, E.; Steinke-Lange, V.; Möslein, G.; Nielsen, M.; Broeke, S.T.; et al. The “unnatural” history of colorectal cancer in Lynch syndrome: Lessons from colonoscopy surveillance. *Int. J. Cancer* **2021**, *148*, 800–811. [[CrossRef](#)]