***Original Article***

# Risk of Colorectal and Gynecologic Cancer in Varied Degree Relatives of Lynch Syndrome and Lynch-like Syndrome Families

Chia-Chen Hsu[1]
Ya-Tin Kuo[1]
Yueh-Chen Lin[1]
Chun-Kai Liao[1]
Yu-Jen Hsu[1]
Jy-Ming Chiang[1,2]
[1]*Colorectal Surgery Section, Department of Surgery, Chang Gung Memorial Hospital,*
[2]*College of Medicine, Chang Gung University, Taoyuan, Taiwan*

***Key Words***
Lynch syndrome
HNPCC
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***Background.*** Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant genetic disorder caused by a variation in one of four DNA mismatch repair genes; such variations increase the risk of several cancers, including colon and endometrial cancer. However, limited research has examined the cancer risk among relatives of patients with HNPCC. This study analyzed the cancer risk among first-degree, second-degree, and third-degree relatives of patients with HNPCC.

***Methods.*** In this study, 50 patients with HNPCC, from 42 unrelated families, who met the Amsterdam-II criteria underwent testing for mismatch repair (MMR) expression; testing was conducted using next-generation sequencing for germline variations and immunohistochemical staining for MMR expression. Pedigree charts were constructed for families spanning at least three generations. The study population was then analyzed for clinical and histological features, pathogenic germline variations, and the rate of associated cancers among relatives of varying degrees of patients with Lynch and Lynch-like syndrome.

***Results.*** Among 715 identified relatives (225 first-degree, 291 second-degree, and 199 third-degree relatives), significant differences were observed in the rates of associated cancers across the three degrees of relationship ($p < 0.001$). The most common cancer types were colorectal and gynecologic cancers. Significant differences were observed in the rates of colorectal cancer ($p < 0.001$) and gynecologic cancer ($p < 0.001$) among first-degree, second-degree, and third-degree relatives. The cumulative incidence risk of colorectal cancer significantly differed between first- and second-degree relatives ($p = 0.001$), but the risk of gynecologic cancer was significantly different between first-degree and third-degree relatives ($p = 0.026$).

***Conclusion.*** This study demonstrated that in Lynch syndrome and Lynch-like syndrome patients, the incidence of colorectal cancer is significantly higher in first-degree relatives compared to second- and third-degree relatives. Moreover, in the case of gynecological cancer, the incidence rate is significantly higher in first-degree relatives than in third-degree relatives, but no significant difference is observed compared to second-degree relatives.

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Correspondence to: Dr. Jy-Ming Chiang, Colorectal Surgery Section, Department of Surgery, Chang Gung Memorial Hospital, No. 5, Fu-Hsing Rd., Kuei-Shan Township, Taoyuan 333, Taiwan. Tel: 886-3-328-1200 ext. 2101; Fax: 886-3-328-5818; E-mail: chunkai.liao@gmail.com

Hereditary nonpolyposis colorectal cancer (HNPCC) is clinically diagnosed on the basis of the Amsterdam-II criteria (A-II-C). Most patients with HNPCC exhibit deficient mismatch repair (dMMR) gene expression. Of these patients with dMMR/HNPCC, most have Lynch syndrome (LS) if germline MMR gene variations are found. However, the condition of other patients is referred to as Lynch-like syndrome (LLS) if they exhibit other genes not related to MMR or no germline variation is identified. The remaining HNPCC cases, which do not involve a loss of MMR protein, are classified as familial colorectal cancer syndrome type X (FCCTX). Studies have identified different clinical characteristics for these subtypes of HNPCC.[1-3]

Families of patients with LS have an increased lifetime risk of cancers, including colorectal cancer (CRC); endometrial cancer; stomach cancer; ovarian cancer; cancers of the small intestine, biliary tract, and brain; and carcinoma of the ureters and renal pelvis.[4-6] Although some studies have indicated that early colonoscopy screening may be beneficial for first-degree relatives and possibly second-degree relatives of patients with LS, whether relatives of different degrees should be treated differently remains unclear.[5,7] In addition, the rates of extracolonic cancers among different degrees of relatives must be clinically clarified because reducing morbidity and mortality from these cancers may be possible through adequate screening and surveillance.[8] Furthermore, understanding the conditions of families of patients with LS can help to identify individuals who may benefit from preventive interventions.

Clinically, family history is a crucial and easily accessible information to inform the management of patients with hereditary cancer and their relatives.[9] Analyzing families by pedigree is a low-cost, noninvasive genetic method for tracking and identifying a familial cancer predisposition.[10] Even in genetic risk assessment and molecular genetic testing, considering a patient's family history is sometimes important for making the correct diagnosis, such as in the case of variants of uncertain significance.[11] Therefore, such family history analysis can help in genetic counseling.

In this study, we investigated the molecular genetic differences of patients with HNPCC. In addition, by analyzing their family pedigrees, we explored whether the risk of associated cancers varies with the degree of relative.

## Material and Methods

The Colorectal Cancer Registry of Chang Gung Memorial Hospital was used to identify patients with CRC and from families meeting the A-II-C. All identified patients provided written informed consent, and the study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (CGMH 201801201B0).

### Pedigree construction

Four nursing specialists and one research assistant were trained by the first and corresponding authors to visit patients and their families and collect patient-reported family histories. The information of members of each family with at least three generations was collected for pedigree chart construction.

The pedigree charts included patients and their family history of cancer and degree of relationship with family members affected by malignancies and well as their sex, age, vital status, age at cancer onset, and type of primary cancer. Data were entered into the Colorectal Cancer Registry.

The salient clinical features, such as tumor stage, size, location, histology, grade, and recurrence status, of the index patients of each family were recorded.

### Immunohistochemical staining

Immunohistochemical (IHC) staining of tumor tissues for MLH1, PMS2, MSH2, and MSH6 was conducted on formalin-fixed paraffin-embedded samples in the pathology laboratory, as described by Chiang et al.[12] The analyses included positive controls, and each slide was required to have a sufficient composition of normal and malignant tissues to ensure analytic coherence. A pathologist evaluated all samples in this study for nuclear MMR staining. If any of the tumor cells (> 5%) displayed positive nuclear staining, the

sample was considered positive for the tested protein.

## Germline variation analysis through NGS

Whole blood was drawn from all the participants. Genomic DNA was extracted using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Each DNA sample was checked for purity by using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). All the samples were obtained and used according to the guidelines of this study's institutional approval after the receipt of written consent from the patients.

A 30-gene panel for CRC susceptibility was used. Of the 30 genes, 13 (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*, *TP53*, *MLH3*, *CHEK2*, *CDH1*, *ATM*, *BRCA1*, *BRCA2*, and *RPS20*) were related to nonpolyposis syndrome, 10 genes (*STK11*, *PTEN*, *BMPR1A*, *SMAD4*, *GREM1*, *RNF43*, *BLM*, *GALNT12*, *AKT1*, and *PIK3CA*) were related to nonadenomatous polyposis diseases, and 7 genes (*APC*, *MUTYH*, *POLE*, *POLD1*, *NTHL1*, *AXIN2*, and *CTNNA1*) were related to adenomatous polyposis syndrome. Details of the laboratory procedure have been reported previously.

Raw sequencing data were trimmed of barcoded adapter sequences and filtered for poor signal reads. They were then aligned to the human genome. Variant calling was performed by using the platform-specific pipeline of VariantCaller v5.10 (Life Technologies). Advanced variant annotation was accomplished by uploading the resultant Variant Call Format (VCF) file from VariantCaller to the cloud software package Ion Reporter (Thermo Fisher Scientific) and the web-based software wANNOVAR (Wang Genomics Lab, http://wannovar.wglab.org/). Only variants interpreted as pathogenic or "likely pathogenic" by the NCBI ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) or VarSome (https://varsome.com/) database were considered for further evaluation. All filter-in variants were confirmed through Sanger sequencing.

## MMR gene copy number and methylation status analysis

To evaluate the possibility of copy number changes in MMR genes, multiplex ligation-dependent probe amplification (MLPA) was applied for patients without pathogenic or likely pathogenic germline alterations. SALSA MLPA P003-D1 and SALSA MLPA P072-C1 (MRC-Holland, Amsterdam, Netherlands) were used for *MLH1*/*MSH2* and *MSH6*, respectively. The probe mix used in the (MS) MLPA included probes that tested for sample fragmentation and sufficient digestion. Fragment analysis of both MS-MLPA and MLPA was performed using an ABI3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) at the Institute for Molecular Medicine of Finland Technology Centre, and analysis was conducted using GeneMapper software version 5.0 (Thermo Fisher, Waltham, MA, USA) and Coalyser$^{TM}$ (MRC-Holland, Amsterdam, Netherlands).

To determine methylation changes in the MMR genes of both normal and tumor samples, MS-MLPA was performed using the SALSA MLPAME011-B3 probe mix (MRC-Holland, Amsterdam, Netherlands) according to the manufacturer's instructions.

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## Statistical analysis

All analyses were conducted using SPSS Statistics version 25.0 (IBM, Armonk, NY, USA). Clinicopathological characteristics and categorical variables are presented as frequencies and proportions and were compared using the $\chi^2$ test. Continuous variables were measured as mean and standard deviations and analyzed using Student's t test. All statistical tests were two-tailed, and a $p$ value of $< 0.05$ was considered significant. We used cumulative incidence analysis to study the likelihood of specific cancers developing in the relatives of patients with LS and LLS. This enabled us to assess the cancer risk differences of first-, second-, and third-degree relatives overtime, with consideration given to the influence of other events.

# Results

## Identifying patients fulfilling the Amsterdam-II-criteria for Lynch syndrome

In this study, 50 suspected Lynch syndrome patients were collected from a colorectal cancer registry based on their fulfillment of Amsterdam-II criteria (A-II C), classifying them as hereditary non-polyposis colorectal cancer (HNPCC). These 50 HNPCC patients came from 42 unrelated families, and their clinical manifestations are presented in Table 1.

## Molecular genetic classification of clinically defined HNPCC

The patients were routinely tested for MMR protein expression through IHC staining and by using NGS for germline variations. In total, 36 of 42 (85.7%) families demonstrated dMMR positivity in IHC staining. Of these 36 families, germline mutations were detected in 31 through NGS. As shown in Table 2, these variations consisted of 16 *MLH1*, 6 *MSH2* germline variations, 4 *EPCAM* duplications with or without *MSH* deletion, 1 large *MSH* deletion, and 1 *MSH* duplication. Three (7.1%) other genes (*1TP53*, *1POLE*, and *1CHEK2*) were detected in three families. The remaining five (11.9%) families demonstrated dMMR expression without any pathologic variation. Therefore, 31 of 42 (73.8%) HNPCC families were molecularly confirmed to have pathogenic germline variations. In summary, 28 of 42 (66.7%) HNPCC families were confirmed to be LS familes, and 8 (19.0%) HNPCC families were confirmed to be LLS families. Six (14.2%) families were FCCTX families.

## Analysis of associated cancer risks by pedigree chart

We followed-up all 36 unrelated LS and LLS families postoperatively until December 2021. The pedigree chart of each family was reviewed and updated. The diagnosis of various types of associated cancers was recorded. In total, 715 relatives were identified, including 225 first-degree relatives, 291 second-degree relatives, and 199 third-degree relatives. We compared the relative risk of cancer occurrence among the first-, second-, and third-degree relatives of the patients (Table 3).

Among 715 relatives, cancer occurred in 178 indi-

**Table 1.** Clinico-histological features of 50 HNPCC patients

| | |
|---|---|
| Age (years) | |
| Mean | 45.1 |
| Range | 26-72 |
| Sex | |
| Male | 27 |
| Female | 23 |
| Tumor location | |
| Right side | 26 |
| Left side | 14 |
| Rectum | 10 |
| Tumor size (cm) | |
| Mean | 5.6 |
| Range | 0.9-12.5 |
| Tumor differentiation | |
| Well | 7 |
| Moderate | 33 |
| Poor | 10 |
| Tumor histology | |
| Adenocarcinoma | 41 |
| Mucinous | 4 |
| Signet ring cell | 5 |
| Tumor stage | |
| T stage | |
| Tis | 2 |
| T1 | 4 |
| T2 | 7 |
| T3 | 24 |
| T4 | 13 |
| N stage | |
| N0 | 33 |
| N1 | 8 |
| N2 | 9 |
| M stage | |
| M0 | 45 |
| M1 | 5 |
| Tumor recurrence | |
| Yes | 12 |
| No | 38 |
| Follow up | |
| Alive | 47 |
| Dead | 3 |
| Extracolonic tumor | |
| Yes | 12 |
| No | 38 |

**Table 2.** Summary of germline pathogenic mutation among 31 Lynch syndrome families

| Family | Sample | Gene | Coding sequence_mut | Variant type | Variant effect | ClinVar definition | VarSome definition |
|---|---|---|---|---|---|---|---|
| J36 | H41 | MSH2 | c.687del | p.Ala230LeufsTer16 | Frameshift deletion | Pathogenic | Pathogenic |
| J33 | H35 | | c.718del | p.Asp240ThrfsTer6 | Frameshift deletion | Not report | Pathogenic |
| J29 | H29 | | c.1226_1227del | p.Gln409ArgfsTer7 | Frameshift deletion | Pathogenic | Pathogenic |
| J19 | H54, H57 | | c.1437_1438insA | p.Glu480ArgfsTer8 | Frameshift insertion | Not report | Pathogenic |
| J38 | H43, H71 | | c.1661+1G>A | | | Likely pathogenic | Pathogenic |
| J20 | H55 | | c.2087C>T | p.Pro696Leu | Missensemutation | Pathogenic | Pathogenic |
| J01 | H1 | | c.2516A>G | p.His839Arg | Missensemutation | Uncertain significance | Likely pathogenic |
| J02 | H2, H72 | | | | EPCAM+MSH2 duplication | | |
| J13 | H67 | | | | EPCAM duplication + MSH2 deletion | | |
| J15 | H50 | | | | EPCAM duplication + MSH2 deletion | | |
| J10 | H13 | | | | EPCAM duplication | | |
| J31 | H31 | | | | MSH2 large deletion | | |
| J12 | H15, H61 | | | | MSH2 duplication | | |
| J37 | H42 | MLH1 | c.67G>T | p.Glu23Ter | Stopgain | Pathogenic | Pathogenic |
| J23 | H21 | | c.103_104insAA | p.Met35LysfsTer2 | Frameshift insertion | Not report | Pathogenic |
| J06 | H7 | | c.350C>T | p.Thr117Met | Missense mutation | Pathogenic | Pathogenic |
| J07 | H103, H9 | | c.790+1G>A | | | Pathogenic | Pathogenic |
| J17, J03, J09, J11, J27, J26, J34 | H12, H52, H3, H11, H14, H25, H26, H38 | | c.793C>T | p.Arg265Cys | Missense mutation | Pathogenic | Pathogenic |
| J05 | H6, H8 | | c.1038G>T | p.Gln346His | Missense mutation | Pathogenic | Pathogenic |
| J35 | H40 | | c.1482_1483insC | p.Thr495HisfsTer8 | Frameshift insertion | Not report | Likely pathogenic |
| J08 | H10 | | c.1852_1854del | p.Lys618del | Frameshift deletion | Pathogenic | Pathogenic |
| J04 | H5 | | c.2042C>T | p.Ala681Val | Missense mutation | Uncertain significance | Pathogenic |
| J32 | H32 | | c.2101C>A | p.Gln701Lys | Missense mutation | Uncertain significance | Likely pathogenic |
| J19 | H54, H57 | ATM | c.8071C>T | p.Arg2691Cys | Missense mutation | Uncertain significance | Likely pathogenic |
| J18 | H53 | TP53 | c.580C>T | p.Leu194Phe | Missense mutation | Likely pathogenic | Pathogenic |
| J22 | H20 | CHEK2 | c.472del | p.Ile158TyrfsTer10 | Frameshift deletion | Not report | Likely pathogenic |

viduals, including 76 first-degree relatives (38 women, 38 men), 72 second-degree relatives (38 women, 34 men), and 30 third-degree relatives (9 women, 21 men). The frequencies of all associated cancers among the first-, second-, and third-degree relatives were significantly different, at 33.8%, 24.7%, and 15.1% for first-, second-, and third-degree relatives, respectively ($p < 0.001$; Table 3). CRC was observed in 151 patients (84.8%), and gynecologic cancer was observed in 24 patients (11.1%); these were the two most common cancer types, followed by hepatobillary cancer in 8 patients (4.5%), gastric cancer in 7 patients (3.9%), central nervous system cancer in 7 patients (3.9%), and urothelial cancer in 6 patients (3.4%). The proportion of relatives who developed cancer was slightly higher in men (Table 3).

## Comparing rates of various types of associated cancers among relatives of different degrees

The incidence of each cancer among the relatives of different degrees is shown in Table 3. The rates of CRC and gynecologic cancer significantly differed among relatives of different degrees. The risk of developing CRC among first-degree relatives was approximately 1.4-fold higher than that among second-degree relatives and 2.2-fold higher than that among third-degree relatives.

Significant differences in CRC ($p < 0.001$) and gynecologic cancer ($p < 0.001$) rate were found among first-, second-, and third-degree relatives. As shown in Table 3, 33.8%, 18.6%, and 10.6% of first-, second-, and third-degree relatives developed CRC, respectively, and 12.3%, 6.8%, and 0% of first-, second-, and third-degree relatives developed gynecologic cancer, respectively.

However, no significant differences were observed between first- and second-degree relatives for other types of cancers.

## Cumulative incidences of CRC and endometrial cancer

Overall, there was no significant difference in the total number of cancers among relatives at different degrees based on cumulative incidence ($p = 0.127$; Fig. 1 and Table 4). However, the cumulative inci-

**Table 3.** Summary of rate of associated cancers among different degree relatives of Lynch and Lynch-like syndrome patients

| | Total | 1st degree | 2nd degree | 3rd degree | *p* value |
|---|---|---|---|---|---|
| Total relatives No. (M/F) | 715 (349/366) | 225 (111/114) | 291 (145/146) | 199 (93/106) | |
| Relatives with cancer No. (%) (M/F) | 178 (24.9) (97/81) | 76 (33.8) (38/38) | 72 (24.7) (38/34) | 30 (15.1) (21/9) | < 0.001 |
| Types of associated cancer | | | | | |
| Colorectal cancer | 151 | 76 (33.8) | 54 (18.6) | 21 (10.6) | < 0.001 |
| Gynecologic cancer* | 24 | 14 (12.3) | 10 (6.8) | 0 (0) | < 0.001 |
| Gastric cancer | 7 | 3 (1.3) | 2 (0.7) | 2 (1.0) | 0.890 |
| Hepatobiliary cancer | 8 | 1 (0.4) | 4 (1.4) | 3 (1.5) | 0.555 |
| CNS tumors | 7 | 2 (0.9) | 3 (1.0) | 2 (1.0) | 1.000 |
| Urothelial cancer | 6 | 2 (0.9) | 3 (1.0) | 1 (0.5) | 0.885 |
| Breast cancer | 3 | 1 (0.4) | 1 (0.3) | 0 (0) | 1.000 |
| Lung cancer | 2 | 1 (0.4) | 0 (0) | 0 (0) | 0.593 |
| Esophageal cancer | 2 | 0 (0) | 2 (0.7) | 0 (0) | 0.341 |
| Leukemia | 1 | 0 (0) | 1 (0.3) | 1 (0.5) | 0.743 |
| Thymus cancer | 1 | 1 (0.4) | 0 (0) | 0 (0) | 0.593 |
| Small bowel cancer | 2 | 1 (0.4) | 1 (0.3) | 0 (0) | 1.000 |
| Pancreatic cancer | 1 | 0 (0) | 0 (0) | 1 (0.5) | 0.278 |
| Prostate cancer | 1 | 0 (0) | 1 (0.3) | 0 (0) | 1.000 |
| Bone cancer | 1 | 0 (0) | 1 (0.3) | 0 (0) | 1.000 |
| Lymphoma | 1 | 0 (0) | 1 (0.3) | 0 (0) | 1.000 |
| Head and neck cancer | 1 | 0 (0) | 0 (0) | 1 (0.5) | 0.278 |
| Others | 2 | 1 (0.4) | 0 (0) | 1 (0.5) | 0.278 |

* Gynecologic cancer included endometrial cancer, ovarian cancer, cervical cancer.
CNS, Central Nervous System.

**Table 4.** Cumulative incidence of age for specific cancer amid Lynch and Lynch-like syndrome

| Cancer type | Degree of relatives | Cumulative risk (%) by age (years) | | | | | | | *p* value |
|---|---|---|---|---|---|---|---|---|---|
| | | 0-20 | 21-30 | 31-40 | 41-50 | 51-60 | 61-70 | 71-80 | |
| All cancers | 1st | 0.5 | 2.0 | 11.1 | 26.7 | 44.6 | 55.1 | 64.1 | 0.127 |
| | 2nd | 0 | 1.9 | 7.1 | 20.2 | 34.5 | 47.5 | 51.4 | |
| | 3rd | 0 | 6.8 | 12.5 | 21.3 | 31.3 | 38.9 | 45.0 | |
| Colorectal cancer | 1st | 0.5 | 2.0 | 10.6 | 24.3 | 39.0 | 50.9 | 60.7 | 0.004 |
| | 2nd | 0 | 1.9 | 6.1 | 14.4 | 22.7 | 32.6 | 36.8 | |
| | 3rd | 0 | 5.0 | 9.7 | 16.6 | 22.5 | 31.1 | 31.1 | |
| Gynecologic cancer | 1st | 0 | 0 | 1.1 | 3.9 | 17.0 | 29.3 | 29.3 | 0.053 |
| | 2nd | 0 | 0 | 0 | 3.2 | 13.9 | 13.9 | 16.7 | |
| | 3rd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Gastric cancer | 1st | 0 | 0 | 0 | 0.8 | 3.3 | 3.3 | 3.3 | 0.557 |
| | 2nd | 0 | 0 | 0 | 0 | 2.1 | 3.8 | 3.8 | |
| | 3rd | 0 | 0 | 0 | 0 | 0 | 5.0 | 14.5 | |
| Hepatobiliary cancer | 1st | 0 | 0 | 0 | 0.7 | 0.7 | 0.7 | 0.7 | 0.530 |
| | 2nd | 0 | 0 | 0 | 2.0 | 2.0 | 3.2 | 3.2 | |
| | 3rd | 0 | 0 | 0 | 0 | 2.6 | 2.6 | 2.6 | |
| Urothelial cancer | 1st | 0 | 0 | 0 | 0 | 0 | 2.2 | 9.2 | 1.000 |
| | 2nd | 0 | 0 | 0 | 0 | 1.0 | 2.7 | 6.9 | |
| | 3rd | 0 | 0 | 0 | 0 | 3.2 | 3.2 | 3.2 | |
| CNS tumors | 1st | 0 | 0 | 0 | 0.7 | 0.7 | 3.7 | 3.7 | 0.680 |
| | 2nd | 0 | 0 | 0.5 | 0.5 | 1.4 | 2.6 | 2.6 | |
| | 3rd | 0 | 1.0 | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | |

CNS, Central Nervous System.

dence of tumor occurrence for all ages among first-degree relatives was marginally significantly higher than that among second-degree relatives ($p = 0.054$; Fig. 1).

Further, as shown in Fig. 2 and Table 4, the risk of CRC significantly differed between first-degree and second-degree relatives ($p = 0.001$) and first-degree and third-degree relatives ($p = 0.051$). However, the risk of gynecologic cancer differed significantly only between first-degree and third-degree relatives ($p = 0.026$; Fig. 3; first-degree vs. second-degree relatives, $p = 0.176$). For both colorectal and gynecologic cancers, no significant difference was observed between second-degree and third-degree relatives ($p = 0.912$ and $p = 0.092$, respectively).

## Discussion

This combined pedigree analysis and molecular genetic test study of families of patients with HNPCC revealed dMMR in 36 of 42 (85.8%) unrelated HNPCC families. Of them, 28 (66.7%) families had pathogenic variations of MMR genes. Of the remaining eight families (19.0%), three exhibited *TP53*, *POLE*, and *CHEK2* variations, but five families did not exhibit any germline variation — these families were considered to have LLS. Six families (14.3%) were considered to have FCCTX.

Pedigree analysis revealed the first-degree to third-degree relatives to have significantly different risks of LS-associated cancer. Colorectal and gynecologic cancers were the two most common types of cancer, followed by liver, stomach, brain, and urinary tract tumors. Moreover, significantly higher risks of CRC were observed among first-degree relatives than second- and third-degree relatives; however, a significantly higher risk of gynecologic cancer was only found among first-degree relatives compared with third-degree relatives. Although the relatives of patients with LS have been reported to be at higher risks of associated cancers, few studies have explored whether differences exist between degrees of relatives.[10] In this study, we determined that cancer type and degree of

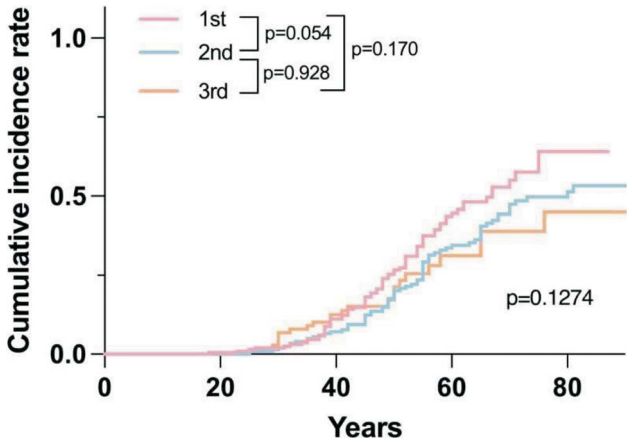


**Fig. 1.** Cumulative risk of all types of cancer related to different degree relatives of Lynch and Lynch-like syndrome patients.

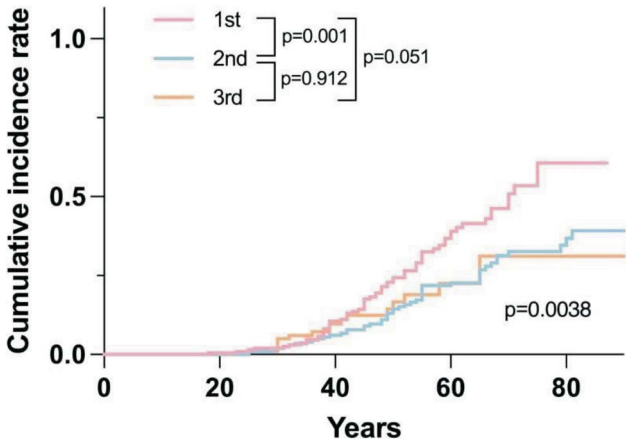


**Fig. 2.** Cumulative risk of colorectal cancer related to different degree relatives of Lynch and Lynch-like syndrome patients.

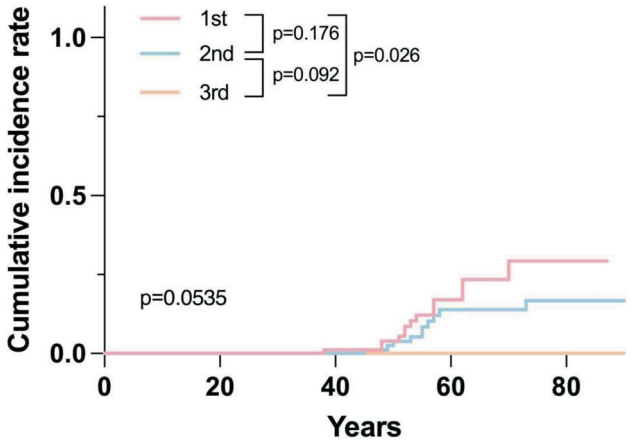


**Fig. 3.** Cumulative risk of gynecologic cancer related to different degree relatives of Lynch and Lynch-like syndrome patients.

relative are associated. Our results highlight that screening policies aimed at identifying individuals at risk should be tailored according to the degree of relative and type of associated cancer.

Combining family history and MMR IHC staining was effective. Globally, including in Taiwan, genetic testing is not a popular means of assessment; however, taking family history in the clinical setting is feasible and practical, as demonstrated in this study. Differentiating LS from FCCTX has profound implications for the cancer risk and surveillance of affected patients and their at-risk relatives.[11,13-15] In this study, we determined that 14.3% of patients with HNPCC had FCCTX, and these patients should be treated through a different strategy than that specified in LS guidelines. Therefore, using family history together with IHC tumor staining can help to differentiate the clinical mimicry within HNPCC and facilitate diagnosis and management.

Early colonoscopy screening is typically recommended for first-degree relatives of Lynch syndrome patients.[15,16] Although, our study revealed a significant increase in the rate of colorectal cancer among first-degree relatives compared to second and third-degree relatives. We suggest that early screening may also be beneficial for second and third-degree relatives, as our data indicates a substantial cumulative incidence of colorectal cancer in these groups.

Regarding the cumulative incidence of gynecologic cancer, the incidence among second-degree relatives was similar to that among first-degree relatives, whereas that among third-degree relatives was low. However, these data need to be interpreted carefully because of the limited case numbers. Although cancer management has been predominantly focused on the individuals affected by disease, not on their families, we propose that better management may be achieved by including family screening practices in care policies. Therefore, knowledge of the risks of various types of cancers among different degrees of relatives could enhance family screening.

In this study, three non-MMR related gene fami-

lies were present with dMMR tumors. Similar results revealed that some non-MMR genes may contribute to the dMMR pattern, thus indicating that a multigene panel should be adopted for assessing dMMR cases to improve the rate of detection of germline variations. Moreover, some early-onset CRCs may arise from other types of hereditary tumor syndromes, such as Li-Fraumeni syndrome, as also shown in this study.

This study explored the risks of colorectal and gynecologic cancers among a diverse range of relatives within families of patients with LS and LLS. CRC and gynecologic cancer were the most prevalent cancer types in this context. Notably, significant differences were observed in their incidence between first-, second-, and third-degree relatives (both $p < 0.001$).

In the overall analysis, cumulative cancer incidence between relatives of varying degrees did not differ significantly ($p = 0.127$). However, across all ages and tumors, first-degree relatives had a marginally higher incidence than did second-degree relatives ($p = 0.054$).

Moreover, the risk of CRC differed significantly between first-degree relatives and second-degree relatives ($p = 0.001$) and between first-degree and third-degree relatives ($p = 0.051$). By contrast, the risk of gynecologic cancer differed significantly only between first-degree relatives and third-degree relatives ($p = 0.026$; first-degree vs. second-degree relatives, $p = 0.176$). No statistically significant differences were found in the risk of colorectal or gynecologic cancer between second-degree and third-degree relatives ($p = 0.912$ and $p = 0.092$, respectively).

Although there's some evidence suggesting a higher risk of cervical cancer in individuals with mutations in MMR genes, it's not currently classified as a cancer associated with Lynch syndrome. However, there is still debate regarding whether cervical cancer should be considered a Lynch syndrome-associated cancer. One study highlighted that women with Lynch syndrome face a six-fold higher risk of cervical cancer compared to the general population, as indicated by data from international registries.[17] Other study suggested non-HPV-associated cervical adenocarcinoma (NHPVA) are diseases related to hereditary genetic factors.[18]

This retrospective report highlights the challenge in accurately pinpointing the location of gynecological cancers during patient interviews, such as distinguishing between lower uterine, endocervical, or purely cervical cancers. The aim of this paper is to alert clinicians that, when monitoring patients and their families for Lynch syndrome, attention should be given to cervical cancer, especially cases unrelated to HPV.

Our study has several limitations. First, the cancer diagnoses were based on NGS of a 30-gene-panel, and some rare germline variations may not have been tested for. Second, given that family cancer history is dynamic, assessment at different times may affect the results of risk analyses.

In conclusion, this study clearly indicates that in LS and LLS patients, the incidence of colorectal cancer is significantly higher in first-degree relatives compared to second- and third-degree relatives. In the case of gynecological cancer, the incidence rate is significantly higher in first-degree relatives than in third-degree relatives, but no significant difference is observed compared to second-degree relatives. It's important to note the substantial rates of colorectal and gynecological cancer among second-degree and third-degree relatives of LS and LLS patients.

## References

1. Lee V, Murphy A, Le DT, Diaz LA Jr. Mismatch repair deficiency and response to immune checkpoint blockade. *Oncologist* 2016;21:1200-11.
2. Buchanan DD, Tan YY, Walsh MD, Clendenning M, Metcalf AM, Ferguson K, Arnold ST, Thompson BA, Lose FA, Parsons MT, Walters RJ, Pearson SA, Cummings M, Oehler MK, Blomfield PB, Quinn MA, Kirk JA, Stewart CJ, Obermair A, Young JP, Webb PM, Spurdle AB. Tumor mismatch repair immunohistochemistry and DNA MLH1 methylation testing of patients with endometrial cancer diagnosed at age younger than 60 years optimizes triage for population-level germline mismatch repair gene mutation testing. *J Clin Oncol* 2014; 32:90-100.
3. Dixon K, Asrat MJ, Bedard AC, Binnington K, Compton K, Cremin C, Heidary N, Lohn Z, Lovick N, McCullum M, Mindlin A, O'Loughlin M, Petersen T, Portigal-Todd C, Scott J, St-Martin G, Thompson J, Turnbull R, Mung SW, Hong Q, Bezeau M, Bosdet I, Tucker T, Young S, Yip S, Aubertin G, Blood KA, Nuk J, Sun S, Schrader KA. Integrating tumor se-

quencing into clinical practice for patients with mismatch repair-deficient Lynch syndrome spectrum cancers. *Clin Transl Gastroenterol* 2021;12:e00397.
4. Watson P, Vasen HFA, Mecklin JP, Bernstein I, Aarnio M, Jarvinen HJ, Myrhoj T, Sunde L, Wijnen JT, Lynch HT. The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome. *Int J Cancer* 2008;123:444-9.
5. Moller P, Seppala T, Bernstein I, Holinski-Feder E, Sala P, Evans DG, Lindblom A, Macrae F, Blanco I, Sijmons R, Jeffries J, Vasen H, Burn J, Nakken S, Hovig E, Rodland EA, Tharmaratnam K, de Vos Tot Nederveen Cappel WH, Hill J, Wijnen J, Green K, Lalloo F, Sunde L, Mints M, Bertario L, Pineda M, Navarro M, Morak M, Renkonen-Sinisalo L, Frayling IM, Plazzer JP, Pylvanainen K, Sampson JR, Capella G, Mecklin JP, Moslein G, Mallorca G. Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database. *Gut* 2017;66:464-72.
6. FC DAS, Wernhoff P, Dominguez-Barrera C, Dominguez-Valentin M. Update on hereditary colorectal cancer. *Anticancer Res* 2016;36:4399-405.
7. Stoffel EM, Kastrinos F. Familial colorectal cancer, beyond Lynch syndrome. *Clin Gastroenterol Hepatol* 2014;12:1059-68.
8. Mukama T, Kharazmi E, Xu X, Sundquist K, Sundquist J, Brenner H, Fallah M. Risk-adapted starting age of screening for relatives of patients with breast cancer. *JAMA Oncol* 2020;6:68-74.
9. Henry T, Lynch TS, Lynch J. An update of HNPCC (Lynch syndrome). *Cancer Genet Cytogenet* 1997;93(1):84-99.
10. Samadder NJ, Smith KR, Wong J, Thomas A, Hanson H, Boucher K, Kopituch C, Cannon-Albright LA, Burt RW, Curtin K. Cancer risk in families fulfilling the Amsterdam criteria for Lynch syndrome. *JAMA Oncol* 2017;3:1697-701.
11. Floria-Santos M, Lopes-Junior LC, Alvarenga Lde M, Ribeiro MS, Ferraz VE, Nascimento LC, Pereira-da-Silva G. Self-reported cancer family history is a useful tool for identification of individuals at risk of hereditary cancer predisposition syndrome at primary care centers in middle-income settings: a longitudinal study. *Genet Mol Biol* 2016;39:178-83.
12. Chen JR, Chiang JM, Changchien CR, Chen JS, Tang RP, Wang JY. Mismatch repair protein expression in Amsterdam II criteria-positive patients in Taiwan. *Br J Surg* 2008;95:102-10.
13. Carethers JM, Stoffel EM. Lynch syndrome and Lynch syndrome mimics: the growing complex landscape of hereditary colon cancer. *World J Gastroenterol* 2015;21:9253-61.
14. Ochs-Balcom HM, Kanth P, Cannon-Albright LA. Early-onset colorectal cancer risk extends to second- and third-degree relatives. *Cancer Epidemiol* 2021;73:101973.
15. Leclerc J, Vermaut C, Buisine MP. Diagnosis of Lynch syndrome and strategies to distinguish Lynch-related tumors from sporadic MSI/dMMR tumors. *Cancers (Basel)* 2021;13.
16. Elsayed FA, Kets CM, Ruano D, van den Akker B, Mensenkamp AR, Schrumpf M, Nielsen M, Wijnen JT, Tops CM, Ligtenberg MJ, Vasen HF, Hes FJ, Morreau H, van Wezel T. Germline variants in POLE are associated with early onset mismatch repair deficient colorectal cancer. *Eur J Hum Genet* 2015;23:1080-4.
17. Antill YC, Dowty JG, Win AK, Thompson T, Walsh MD, Cummings MC, Gallinger S, Lindor NM, Le Marchand L, Hopper JL, Newcomb PA, Haile RW, Church J, Tucker KM, Buchanan DD, Young JP, Winship IM, Jenkins MA. Lynch syndrome and cervical cancer. *Int J Cancer* 2015;137:2757-61.
18. Carvalho JP, Carvalho FM, Chami AM, Filho A, Primo W. Hereditary determinants of gynecological cancer and recommendations. *Rev Bras Ginecol Obstet* 2021;43:638-43.

**原　著**

# 林奇氏症候群和類林奇氏症候群家族之不同程度親屬之大腸癌和婦科癌症的風險

許嘉宸[1]　郭雅婷[1]　林岳辰[1]　廖俊凱[1]　許祐仁[1]　江支銘[1,2]

[1]林口長庚紀念醫院　大腸直腸外科

[2]長庚大學　醫學院

**目的**　遺傳性非息肉狀結直腸癌 (HNPCC) 是一種染色體顯性遺傳疾病，由四種 DNA 錯配修復基因中的變異引起；這些變異會增加多種癌症的風險，包括大腸直腸和子宮內膜癌等。然而目前對於 HNPCC 患者不同親等間親屬的癌症罹病風險研究有限。本研究分析了 HNPCC 患者的第一、二、三等親屬間的癌症罹病風險。

**方法**　在本篇研究中，共有 50 位 HNPCC 患者符合 Amsterdam criteria-II，其來自於不同的 42 個家庭。所有病患皆接受 DNA 錯配修復基因檢測，其使用次世代基因檢測及免疫組織化學染色法。研究中繪製了各病患的家族譜，紀錄至少第一、二、三等親屬間的罹病情形，近一步分析不同親等間的罹病風險。

**結果**　共計 715 位親屬 (225 位一等親，291 位二等親，199 位三等親) 納入了本研究，統計顯示不同親等間林奇氏症候群相關癌症的發病率存在顯著差異。其中最常見的癌症為大腸直腸癌及婦科癌症。在不同親等間，大腸直腸癌和婦科癌症的發病率也存在顯著差異。大腸直腸癌的累積發病風險在第一、二等親屬之間有顯著差異，而婦科癌症的風險在第一、三等親屬之間有顯著差異。

**結論**　本篇文章旨在提供臨床醫師在診治林奇氏症候群患者及其親屬時，進一步了解不同程度親屬之癌症罹病的風險。

**關鍵詞**　林奇氏症候群、遺傳性非息肉狀結直腸癌、大腸癌、大腸直腸癌。