*Original Article*

# Differences of the Clinicopathological Features and Expression of Mismatch Repair Proteins between Colorectal Cancer Patients Who Fulfilled with the Amsterdam-II Criteria Completely and Lack Only One

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***Key Words***
Amsterdam II criteria;
Detect mismatch repair gene expression;
Metachronous CRC;
Male;
Poor differentiation

***Purpose.*** Hereditary nonpolyposis colorectal cancer (HNPCC) patients were reported with clinicopathological characteristics. To compare the clinicopathological characteristics of patients who satisfied the Amsterdam II criteria (A-II C) or lack one criterion alone (HNPCC-like).

***Methods.*** Immunohistochemistry was used to detect mismatch repair (MMR) gene expression. Cox proportional hazards model was used to investigate the effect of the A-II C and MMR status on survival and clinicpathological factors.

***Results.*** We retrospectively evaluated patients who satisfied the A-II C or lack one criterion alone over a period of 14 years. 380 CRC patients were collected including 177 patients with HNPCC and 203 HNPCC-like cases (lacking one A-II criterion) were analyzed. Overall, 63.3% of the HNPCC patients and 16.3% of the HNPCC-like cases demonstrated loss of at least one MMR protein. MMR-deficient (dMMR) patients had larger tumors (28 $cm^2$ vs. 18 $cm^2$, $p < 0.0001$), deeper (T4) tumor invasion (40.7% vs. 29.0%, $p < 0.0173$), lower rates of lymph node involvement (N0, 31.0% vs. 48.5%, $p = 0.0034$), and fewer distant metastases (M0, 8.3% vs. 15.3%, $p = 0.0447$) than MMR-proficient (pMMR) patients. The dMMR/HNPCC-like subgroup also had significantly more male patients (72.7% vs. 43.8%, $p = 0.0034$) and a higher rate of poor differentiation (42.4% vs. 22.9%, $p < 0.028$) than the dMMR/HNPCC subgroup. Significantly different rates of developing metachrous CRC were observed, ranging from lowest 3.28 (pMMR/HNPCC-like), 6.18 (pMMR/HNPCC), 20.57 (dMMR/HNPCC), to highest 37.78 person-years.

***Conclusion.*** We reported distinguishing features related to the subgroups of dMMR/HNPCC-like patients, including male predominance and an extremely high rate of poor differentiation. In addition, risk of developing metachronous CRC might be further classified by combining family history and MMR status.

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Hereditary nonpolyposis colorectal cancer (HNPCC) is clinically defined based on family history, using the Amsterdam-II criteria (A-II C). Up to 60% of families with HNPCC have germ-line mismatch repair (MMR) gene mutations and are diagnosed with Lynch syndrome (LS). To improve a substantial detection rate of patients with LS, reflex tumor tissue testing of all colorectal cancer (CRC) patients for LS by using immunohistochemistry (IHC), which recently emerged as an efficient tool for the detection of MMR abnormalities in resected cancer specimens, has been proposed.[1,2] This method is feasible for the early detection of LS; however, the world-wide adopting routine IHC analysis for all resected CRC tumor tissues to detect the loss of MMR protein expression remains unclear.[3]

HNPCC or LS patients were reported to have some clinicopathological characteristics including higher risks of metachronous CRC,[4] younger age at onset, right side colon predominance, higher proportion of poorly differentiated and mucinous adenocarcinoma,[5] and a specific tumor lymphocyte infiltration pattern[6] although these features could not be distinguished from sporadic tumors with microsatellite instability (MSI).[7] Moreover, these histopathologic characteristics highly suggestive of LS, but not shared by familial colorectal cancer type X, were recently reported.[8]

Clinically, family history is of paramount importance because it is not only one part of basic information but also serves as a risk factor, and necessitates the modification of CRC management, which in turn might also affect survival.[9,10] Based on family history, clinicians might easily identify some hereditary cases and adjust their medical management in terms of surveillance and follow-up to improve outcomes. Furthermore, this information would provide oncologists with the opportunity to assess newly defined cancer susceptibility genes and/or more advanced genetic testing.

In this study, by combining detailed family history with IHC testing for MMR protein expression, we retrospectively analyzed patients who fulfilled the A-II C or A-II-like criteria. In total, 380 CRC patients who underwent surgical resection were included. We compared the clinicopathological characteristics, treatment outcomes, and risks of developing metachronous CRC between different patient subgroups.

## Patients and Method

### Registry and patients

We established the CRC Registry in 1985 in Chang Gung Memorial Hospital (CGMH), and a revised computerized data record form was implemented in 1995. Our database included records of detailed family histories, demographic variables, preoperative evaluation, surgery, and postoperative follow-up.[11] Family history was recorded by tracing pedigrees backward and laterally as far as possible. Patients fulfilling the A-II C (at least three relatives with a Lynch-associated cancer, one being a first-degree relative of the other two; at least two successive generations affected; and at least one person diagnosed before 50 years of age) were defined as HNPCC patients, and those not satisfying only one criterion of the A-II C were defined as HNPCC-like patients.

Between January 1995 and December 2012, 14479 patients were screened. Of these, 380 patients fulfilled the A-II C or lacked only one criterion from the A-II C. Patient data were retrieved from the CRC Registry of CGMH. This study was approved by the institutional review board (IRB) of CGMH (IRB102-2284B).

### IHC analysis for MMR gene expression

Paraffin-embedded tumor blocks from HNPCC and HNPCC-like patients were retrieved from the Pathology Department of CGMH. For each patient, 4-μm thick sections from one formalin-fixed, paraffin-embedded tissue block containing both tumor tissue and normal adjacent mucosa were obtained. Immunostaining was performed on a Dako Universal Autostainer (DakoCytomation, Denmark) by using ChemMateTM EnvisonTM + Detection kits (DakoCytomation, Denmark) in accordance with the manufacturer's instructions. Deparaffinization and rehydration were performed using xylene and graded alcohol. Heat-induced antigen retrieval was performed by immersing the slides in 10 mM citrate buffer (pH 6) at

120 °C for 10 minutes in a pressurized heating chamber (Biocare Medical, Concord, CA). Endogenous peroxidase was blocked using 3% aqueous hydrogen peroxide, and nonspecific binding was blocked using 20% Protein Blocker (Signet Laboratories, Dedham, MA) in Tris buffered saline. Sections were incubated at 4 °C overnight with mouse monoclonal antibodies against hMLH1 (clone G168-728, BD PharMingen, San Diego, CA) at a 1:50 dilution, hMSH2 (clone FE 11, Oncogene Research Products, Cambridge, MA) at a 1:100 dilution, hMSH6 (clone 44, BD PharMingen, San Diego, CA) at a 1:200 dilution, and hPMS2 (clone A16-4, BD PharMingen, San Diego, CA) at a 1:100 dilution. Signal detection was performed using 3,3'-diaminobenzidine as the chromogen. The slides were counterstained with hematoxylin, cleared in xylene, and mounted with Permount. For the negative controls, the primary antibody was replaced with PBS.

### Assessment of MMR gene expression

For the evaluation of IHC results, abnormal staining was defined as total loss of protein in the tumor, using appropriate controls; staining was considered assessable when the nucleus was stained in cells serving as internal controls, including either stromal or germinal follicle lymphocytes or normal epithelial cells in the crypt bases. Tumors were considered negative for MMR protein expression when neoplastic cells showed complete absence of detectable nuclear staining in a sample for which internal positive controls were stained (Fig. 3). Heterogeneous positive or complete positive nuclear staining in tumor samples indicated positive MMR protein expression, whereas weak staining in < 5% of the sample indicated negative MMR protein expression. Other heterogeneous staining patterns in tumors indicated positive MMR protein expression. A pathologist (T-C Chen), who had no knowledge of the family history or other clinicopathological features, reviewed all cases to confirm the immunostaining results.

### Statistical analyses

Pearson's chi-square, Fisher's exact, and Wilcoxon rank-sum tests were used to evaluate the distribution of patient characteristics between five family history groups. Survival curves were estimated using the Kaplan-Meier method. In univariate survival analysis, the associations between patient characteristics and disease-free survival (DFS) and overall survival (OS) were evaluated using the log-rank test. The Cox proportional hazards model was used to investigate the effect of family history groups and MMR status on survival while adjusting for other explanatory variables.

The rate of metachronous CRC was calculated as the number of secondary cancers divided by the number of observed person-years during the follow-up period. In order to explore the association between family history groups and risk of secondary cancer occurrence, the risk ratio of cumulative incidence of secondary malignancies was also estimated using the Cox proportional hazards model. In Cox regression, a patient who died during the follow-up period and who did not develop second malignancy was treated as a censored case. All statistical analyses were performed using the SPSS 17 software (SPSS, Inc., Chicago, IL). The $p$-values were two-sided and those $< 0.05$ were considered statistically significant.

## Results

Between January 1995 and December 2012, 14479 patients were screened. Of these, 380 patients, with 177 HNPCC (fulfilling the A-II C), and 203 HNPCC-like patients (lacking only one criterion of A-II C defined as a group of "Amsterdam-II minus one criterion"), were included in this study.

### Comparisons between HNPCC and HNPCC-like patients

Patients with HNPCC and HNPCC-like disease differed significantly in their clinicopathological features (Table 1). HNPCC patients showed higher rates of loss of MMR gene expression (63.3% vs. 16.3%), younger age at diagnosis (50.4 vs. 58.9 years), right colon predominance (51.4% vs. 33.0%), fewer male patients (46.9% vs. 57.1%), a higher proportion of mucinous adenocarcinoma (16.8% vs. 9.0%), larger

tumor size (20.0 $cm^2$ vs. 14.4 $cm^2$), more frequent extensive resection (29.4% vs. 9.8%), and fewer distant metastases (8.5% vs. 16.3%) than patients with HNPCC-like disease. The distribution of the loss of MMR gene expression did not differ between the HNPCC and HNPCC-like groups, that is , the concordant losses of MLH1/PMS2 staining (69.0% vs. 70.6%) and MSH2/MSH6 staining (27.6% vs. 26.5%) were similar between the HNPCC and HNPCC-like patients, respectively. Discordant loss patterns were identified in only 5 cases with loss of PMS2 only (3.7% vs. 2.9%) (Table 1).

**Table 1.** Comparisons of clinicopathological characteristics between HNPCC and HNPCC-like patients

| Characteristics | Family history | | *p*-value |
|---|---|---|---|
| | HNPCC | HNPCC-like | |
| | No (%) (N = 177) | No (%) (N = 203) | |
| Loss of MMR gene expression | | | < 0.0001 |
| Yes | 112 (63.3) | 33 (16.3) | |
| No | 65 (36.7) | 170 (83.7) | |
| Age at diagnosis | | | < 0.0001 |
| Mean (SD) | 50.4 (12.9) | 58.9 (13.9) | |
| Median (range) | 49 (26-88) | 59 (27-98) | |
| Sex | | | 0.046 |
| Female | 94 (53.1) | 87 (42.9) | |
| Male | 83 (46.9) | 116 (57.1) | |
| Multiple tumors | | | 0.1385 |
| No | 127 (71.8) | 159 (78.3) | |
| Yes | 50 (28.2) | 44 (21.7) | |
| Operation type | | | < 0.0001 |
| Segmental | 125 (70.6) | 183 (90.2) | |
| Subtotal/total | 52 (29.4) | 20 (9.8) | |
| Tumor location | | | 0.0013 |
| Right colon | 91(51.4) | 67 (33.0) | |
| Left colon | 49 (27.7) | 74 (36.5) | |
| Rectum | 37 (20.9) | 62 (30.5) | |
| Histology | | | 0.0243 |
| Adenocarcinoma | 144 (83.2) | 182 (91.0) | |
| Mucinous/signet ring | 29 (16.8) | 18 (9.0) | |
| Tumor differentiation | | | 0.2967 |
| Well/Moderate | 141 (81.0) | 171 (85.1) | |
| Poor | 33 (19.0) | 30 (14.9) | |
| Tumor area (width × length, $cm^2$) | | | 0.0003 |
| Mean (SD) | 25.12 (19.20) | 19.56 (19.23) | |
| Median (range) | 20.00 (0.36-100.00) | 14.43 (0.12-143.00) | |
| TNM_T | | | 0.1673 |
| 0 | 3 (1.7) | 10 (5.0) | |
| 1 | 10 (5.7) | 21 (10.4) | |
| 2 | 14 (8.0) | 18 (9.0) | |
| 3 | 86 (49.1) | 88 (43.8) | |
| 4 | 62 (35.4) | 64 (31.8) | |
| TNM_N | | | 0.1202 |
| 0 | 112 (64.0) | 107 (53.2) | |
| 1 | 38 (21.7) | 51 (25.4) | |
| 2 | 20 (11.4) | 38 (18.9) | |
| 3 | 5 (2.9) | 5 (2.5) | |
| TNM_M | | | 0.0227 |
| 0 | 162 (91.5) | 170 (83.7) | |
| 1 | 15 (8.5) | 33 (16.3) | |

HNPCC, hereditary nonpolyposis colorectal cancer; MMR, mismatch repair; TNM, tumor node metastasis; SD, standard deviation.

## Comparisons between MMR-deficient and MMR-proficient patients

In addition to having a significantly younger age at diagnosis, higher rate of poor differentiation, more frequent occurrence of mucinous/signet ring cell adenocarcinoma, right colon predominant CRC, and a higher rate of multiple colorectal tumors, MMR-deficient (dMMR) patients also had larger tumors (28 $cm^2$ vs. 18 $cm^2$, $p < 0.0001$), deeper (T4) tumor invasion (40.7% vs. 29.0%, $p < 0.0173$), lower rate of lymph node involvement (N0, 31.0% vs. 48.5%, $p = 0.0034$) and fewer distant metastases (M0, 8.3% vs. 15.3%; $p = 0.0447$; Table 2) than MMR-proficient (pMMR) patients.

## Subgroup comparisons between HNPCC and HNPCC-like patients combing with dMMR or pMMR status

In order to further investigate the differences between the HNPCC and HNPCC-like patients with

**Table 2.** Comparisons of clinicopathological characteristics between patients with or without loss of MMR gene expression

| Characteristics, N (%) | Loss of MMR gene expression | | *p*-value |
|---|---|---|---|
| | Yes; N = 145 | No; N = 235 | |
| Family history | | | < 0.0001 |
| HNPCC | 112 (77.2) | 65 (27.7) | |
| HNPCC-like | 33 (22.8) | 170 (72.3) | |
| Age | | | < 0.0001 |
| Mean (SD) | 48.3 (12.5) | 59.0 (13.5) | |
| Median (range) | 48 (26-78) | 59 (29-98) | |
| Sex | | | 0.535 |
| Female | 72 (49.7) | 109 (46.4) | |
| Male | 73 (50.3) | 126 (53.6) | |
| Multiple tumors | | | < 0.0001 |
| No | 89 (61.4) | 197 (83.8) | |
| Yes | 56 (38.6) | 38 (16.2) | |
| Operation type | | | < 0.0001 |
| Segmental | 99 (68.3) | 209 (88.9) | |
| Subtotal/total | 46 (31.7) | 26 (11.1) | |
| Tumor location | | | < 0.0001 |
| Right colon | 87 (60.0) | 71 (30.2) | |
| Left colon | 36 (24.8) | 87 (37.0) | |
| Rectum | 22 (15.2) | 77 (32.8) | |
| Histology | | | 0.0012 |
| Adenocarcinoma | 114 (80.3) | 212 (91.8) | |
| Mucinous/signet ring | 28 (19.7) | 19 (8.2) | |
| Tumor grade | | | < 0.0001 |
| Well/moderate | 103 (72.5) | 209 (89.7) | |
| Poor | 39 (27.5) | 24 (10.3) | |
| Tumor area (width × length, $cm^2$) | | | < 0.0001 |
| Mean (SD) | 28.7 (21.19) | 18.05 (16.98) | |
| Median (range) | 26.00 (0.56-143.00) | 14.00 (0.12-110.00) | |
| TNM_T | | | 0.0173 |
| 0 | 2 (1.4) | 11 (4.8) | |
| 1 | 6 (4.1) | 25 (10.8) | |
| 2 | 14 (9.7) | 18 (7.8) | |
| 3 | 64 (44.1) | 110 (47.6) | |
| 4 | 59 (40.7) | 67 (29.0) | |
| TNM_N | | | 0.0034 |
| 0 | 100 (69.0) | 119 (51.5) | |
| 1 | 30 (20.7) | 59 (25.5) | |
| 2 | 12 (8.3) | 46 (19.9) | |
| 3 | 3 (2.0) | 7 (3.0) | |
| TNM_M | | | 0.0447 |
| No | 133 (91.7) | 199 (84.7) | |
| Yes | 12 (8.3) | 36 (15.3) | |

dMMR tumors, dMMR/HNPCC and dMMR/HNPCC-like subgroups were compared (Table 3). Significant differences were observed in the sex ratio and tumor differentiation. Compared to dMMR/HNPCC patients, more male patients (72.7% vs. 43.8%, $p$ = 0.0034) and a higher rate of poor differentiation (42.4% vs. 22.9%, $p$ = 0.028) were associated with dMMR/HNPCC-like patients (Table 3). However, there is no significant difference between the pMMR/HNPCC and pMMR/HNPCC-like patients were iden-

**Table 3.** Comparisons of clinic-pathologic characteristics between HNPCC and HNPCC-like patients with or without loss of MMR gene expression

| Characteristics | Loss of MMR gene expression (N = 145) | | | No loss of MMR gene expression (N = 235) | | |
|---|---|---|---|---|---|---|
| | HNPCC-like (N = 33) | HNPCC (N = 112) | $p$-value | HNPCC-like (N = 170) | HNPCC (M = 65) | $p$-value |
| Age | | | 0.5674 | | | 0.0017 |
| Mean (SD) | 49.4 (14.5) | 48.0 (11.2) | | 60.7 (13.1) | 54.6 (13.7) | |
| Median (range) | 51 (27-77) | 47.5 (26-78) | | 61 (33-98) | 52 (29-88) | |
| Sex | | | 0.0034 | | | 0.8035 |
| Female | 9 (27.3) | 63 (56.2) | | 78 (45.9) | 31 (47.7) | |
| Male | 24 (72.7) | 49 (43.8) | | 92 (54.1) | 34 (52.3) | |
| Multiple cancer | | | 0.1854 | | | 0.8397 |
| No | 17 (51.5) | 72 (64.3) | | 142 (83.5) | 55 (84.6) | |
| Yes | 16 (48.5) | 40 (35.7) | | 28 (16.5) | 10 (15.4) | |
| Operation type | | | 0.0199 | | | 0.0766 |
| Segmental/hemicolectomy | 28 (84.9) | 71 (63.4) | | 155 (91.2) | 54 (83.1) | |
| Subtotal/total | 5 (15.1) | 41 (36.6) | | 15 (8.8) | 11 (16.9) | |
| Tumor location | | | 0.2524 | | | 0.4655 |
| Right colon | 18 (54.6) | 69 (61.6) | | 49 (28.8) | 22 (33.8) | |
| Left colon | 7 (21.2) | 29 (25.9) | | 67 (39.4) | 20 (30.8) | |
| Rectum | 8 (24.2) | 14 (12.5) | | 54 (31.8) | 23 (35.4) | |
| Histology | | | 0.8001 | | | 0.353 |
| Adenocarcinoma | 27 (81.8) | 87 (79.8) | | 155 (92.8) | 57 (89.1) | |
| Mucinous/signet ring | 6 (18.2) | 22 (20.2) | | 12 (7.2) | 7 (10.9) | |
| Tumor differentiation | | | 0.028 | | | 0.5307 |
| Well/moderate | 19 (57.6) | 84 (77.1) | | 152 (90.5) | 57 (87.7) | |
| Poor | 14 (42.4) | 25 (22.9) | | 16 (9.5) | 8 (12.3) | |
| Area (width × length, $cm^2$) | | | 0.4254 | | | 0.0784 |
| Mean (SD) | 32.53 (26.04) | 27.69 (19.53) | | 17.04 (16.56) | 20.69 (17.91) | |
| Median (range) | 26.00 (3.36-143.00) | 25.83 (0.56-95.00) | | 12.17 (0.12-110.00) | 16.00 (0.36-100.00) | |
| TNM_T | | | 0.9116 | | | 0.4221 |
| 0/1 | 1 (3.0) | 7 (6.3) | | 20 (11.9) | 5 (7.9) | |
| 2 | 4 (12.1) | 10 (8.9) | | 14 (8.3) | 4 (6.4) | |
| 3 | 13 (39.4) | 51 (45.5) | | 75 (44.6) | 35 (55.6) | |
| 4 | 15 (45.5) | 44 (39.3) | | 49 (29.2) | 18 (28.6) | |
| TNM_N | | | 0.4222 | | | 0.9896 |
| 0 | 20 (60.6) | 80 (71.4) | | 87 (51.8) | 32 (50.8) | |
| 1 | 9 (27.3) | 21 (18.8) | | 42 (25.0) | 17 (27.0) | |
| 2 | 4 (12.1) | 8 (7.1) | | 34 (20.2) | 12 (19.1) | |
| 3 | 0 (0.0) | 3 (2.7) | | 5 (3.0) | 2 (3.2) | |
| TNM_M | | | 0.9999 | | | 0.1091 |
| No | 30 (90.9) | 103 (92.0) | | 140 (82.4) | 59 (90.8) | |
| Yes | 3 (9.1) | 9 (8.0) | | 30 (17.6) | 6 (9.2) | |

HNPCC, hereditary nonpolyposis colorectal cancer; MMR, mismatch repair; TNM, tumor node metastasis; SD, standard deviation.

tified (Table 3). Furthermore, subgroups analysis between MMR-deficient and MMR-proficient patients among HNPCC and HNPCC-like patients showed similar results, that is, having a significantly younger age at diagnosis, higher rate of poor differentiation, more frequent occurrence of mucinous/signet ring cell adenocarcinoma, right colon predominant CRC, and lower rate of lymph node involvement for dMMR than MMR-proficient (pMMR) patients for both HNPCC and HNPCC-like patients (Table 4).

## Risk of metachronous CRC among different subgroups classified by MMR gene expression and A-II C

The rate and cumulative incidence of developing metachronous CRC (m-CRC) among different sub-

**Table 4.** Comparisons of clinic-pathologic characteristics between loss of MMR gene expression or not among HNPCC and HNPCC-like patients

| Characteristics | HNPCC | | | HNPCC-like | | |
|---|---|---|---|---|---|---|
| | pMMR (N = 65) | dMMR (N = 112) | *p* value | pMMR (N = 170) | dMMR (N = 33) | *p* value |
| Age | | | 0.0041 | | | 0.0001 |
| Mean (SD) | 54.6 (13.7) | 48.0 (11.2) | | 60.7 (13.1) | 49.4 (14.5) | |
| Median (range) | 52 (29-88) | 47.5 (26-78) | | 61 (33-98) | 51 (27-77) | |
| SEX | | | 0.2714 | | | 0.0481 |
| Female | 31 (47.7) | 63 (56.2) | | 78 (45.9) | 9 (27.3) | |
| Male | 34 (52.3) | 49 (43.8) | | 92 (54.1) | 24 (72.7) | |
| Multiple tumor | | | 0.0038 | | | < 0.0001 |
| No | 55 (84.6) | 72 (64.3) | | 142 (83.5) | 17 (51.5) | |
| Yes | 10 (15.4) | 40 (35.7) | | 28 (16.5) | 16 (48.5) | |
| Operation type | | | 0.0056 | | | 0.3334 |
| Segmental | 54 (83.1) | 71 (63.4) | | 155 (91.2) | 28 (84.9) | |
| Subtotal/total | 11 (16.9) | 41 (36.6) | | 15 (8.8) | 5 (15.1) | |
| Tumor location | | | 0.0002 | | | 0.0138 |
| Right colon | 22 (33.8) | 69 (61.6) | | 49 (28.8) | 18 (54.6) | |
| Left colon | 20 (30.8) | 29 (25.9) | | 67 (39.4) | 7 (21.2) | |
| Rectum | 23 (35.4) | 14 (12.5) | | 54 (31.8) | 8 (24.2) | |
| Histology | | | 0.116 | | | 0.0869 |
| Adenocarcinoma | 57 (89.1) | 87 (79.8) | | 155 (92.8) | 27 (81.8) | |
| Mucinous/signet ring | 7 (10.9) | 22 (20.2) | | 12 (7.2) | 6 (18.2) | |
| Tumor grade | | | 0.0836 | | | < 0.0001 |
| Well/moderate | 57 (87.7) | 84 (77.1) | | 152 (90.5) | 19 (57.6) | |
| Poor | 8 (12.3) | 25 (22.9) | | 16 (9.5) | 14 (42.4) | |
| Tumor size (width × length $cm^2$) | | | 0.0038 | | | < 0.0001 |
| Mean (SD) | 20.69 (17.91) | 27.69 (19.53) | | 17.04 (16.56) | 32.53 (26.04) | |
| Median (range) | 16.00 (0.36-100.00) | 25.83 (0.56-95.00) | | 12.17 (0.12-110.00) | 26.00 (3.36-143.00) | |
| TMN_T | | | 0.4738 | | | 0.1533 |
| 0 | 1 (1.6) | 2 (1.8) | | 10 (6.0) | 0 (0.0) | |
| 1 | 5 (7.9) | 5 (4.5) | | 20 (11.9) | 1 (3.0) | |
| 2 | 4 (6.4) | 10 (8.9) | | 14 (8.3) | 4 (12.1) | |
| 3 | 35 (55.6) | 51 (45.5) | | 75 (44.6) | 13 (39.4) | |
| 4 | 18 (28.6) | 44 (39.3) | | 49 (29.2) | 15 (45.5) | |
| TMN_N | | | 0.024 | | | 0.6083 |
| 0 | 32 (50.8) | 80 (71.4) | | 87 (51.8) | 20 (60.6) | |
| 1 | 17 (27.0) | 21 (18.8) | | 42 (25.0) | 9 (27.3) | |
| 2 | 12 (19.1) | 8 (7.1) | | 34 (20.2) | 4 (12.1) | |
| 3 | 2 (3.2) | 3 (2.7) | | 5 (3.0) | 0 (0.0) | |
| TMN_M | | | 0.7832 | | | 0.2228 |
| No | 59 (90.8) | 103 (92.0) | | 140 (82.4) | 30 (90.9) | |
| Yes | 6 (9.2) | 9 (8.0) | | 30 (17.6) | 3 (9.1) | |

groups of CRC patients are summarized in Table 5 and Fig. 1. Significantly different rates of m-CRCs were observed among different patient subgroups: from lowest 3.28 person-years (pMMR/HNPCC-like), 6.18 person-years (pMMR/HNPCC), 20.57 person-years (dMMR/HNPCC), to highest 37.78 person-years (dMMR/HNPCC-like). In addition, significantly different cumulative incidences were observed (Table 4 and Fig. 1). Patients in the dMMR/HNPCC-like group had the highest risk (11.0%, 84.8%, and 84.8% for 10, 20, and 30 years, respectively) followed by those in the dMMR/HNPCC group (12.0%, 61.4%, and 86.2% for 10, 20, and 30 years, respectively). Risk comparisons of the cumulative incidences of different dMMR statuses combined with patient subgroups with family history showed that the adjusted hazard ratios (HR) for the dMMR/HNPCC-like (10.02; 95% confidence interval (CI), 3.04-33.00; $p < 0.0001$) and dMMR/HNPCC (5.44; 95% CI, 1.62-18.26; $p = 0.006$) groups were significantly higher than that for pMMR/HNPCC-like group.

## Survival comparisons between different subgroups classified by MMR gene expression and A-II C

The DFS and OS of different patient subgroups classified by MMR gene expression and A-II C were compared (Figs. 2A-2D). Significantly better DFS (HR = 0.329; 95% CI, 0.137-0.792; $p = 0.0132$; Table 6) and OS (HR = 0.439; 95% CI, 0.234-0.824; $p = 0.0104$; Table 6) were observed in dMMR patients compared to pMMR patients. Furthermore, HNPCC

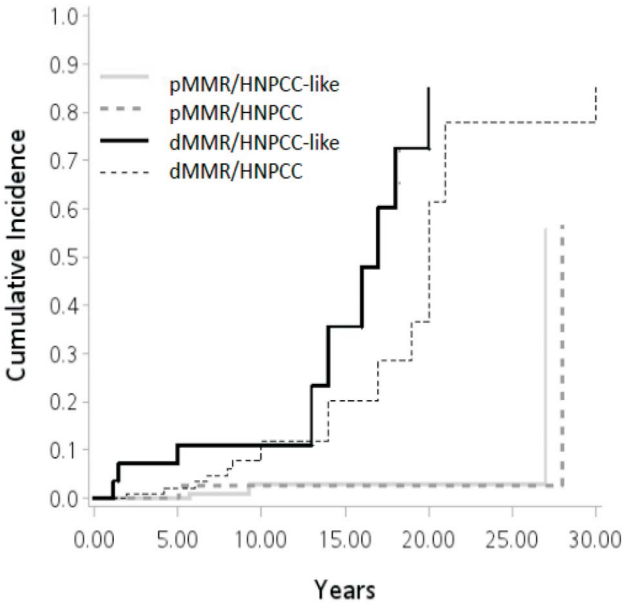


**Fig. 1.** Cumulative incidence of developing metachronous colorectal cancer among different mismatch repair gene expression status and family history subgroups of colorectal cancer patients.

**Table 5.** Rate and cumulative incidence of developing metachronous colorectal cancer among different subgroups of CRC patients

| Family history | MMR sufficient | | MMR deficient | | *p*-value |
|---|---|---|---|---|---|
| | HNPCC-like | HNPCC | HNPCC-like | HNPCC | |
| | 0 (N = 169) | 1 (N = 64) | 2 (N = 28) | 3 (N = 107) | |
| Age [mean ± SD] | 60.2 ± 13.3 | 53.6 ± 13.3 | 46.4 ± 13.5 | 45.8 ± 11.8 | < 0.0001 |
| Sex [N (%)] | | | | | 0.0513 |
| Female | 78 (46.2%) | 31 (48.4%) | 7 (25.0%) | 58 (54.2%) | |
| Male | 91 (53.9%) | 33 (51.6%) | 21 (75.0%) | 49 (45.8%) | |
| Frequency of 2nd CRC | 3 (1.8%) | 2 (3.1%) | 9 (32.1%) | 17 (15.9%) | < .0001 |
| Rate of 2nd CRC [per 1000 person-years] | 3.28 | 6.18 | 37.78 | 20.57 | < .0001 |
| Cumulative incidence | | | | | < .0001 |
| 5-yrs | 0.90% | 2.70% | 11.00% | 2.20% | |
| 10-yrs | 2.80% | 2.70% | 11.00% | 12.00% | |
| 15-yrs | 2.80% | 2.70% | 11.00% | 12.00% | |
| 20-yrs | 2.80% | 2.70% | 84.80% | 61.40% | |
| 25-yrs | 2.80% | 2.70% | 84.80% | 77.90% | |
| 30-yrs | 55.60% | 56.60% | 84.80% | 86.20% | |

HNPCC, hereditary nonpolyposis colorectal cancer; CRC, colorectal cancer; MMR, mismatch repair.

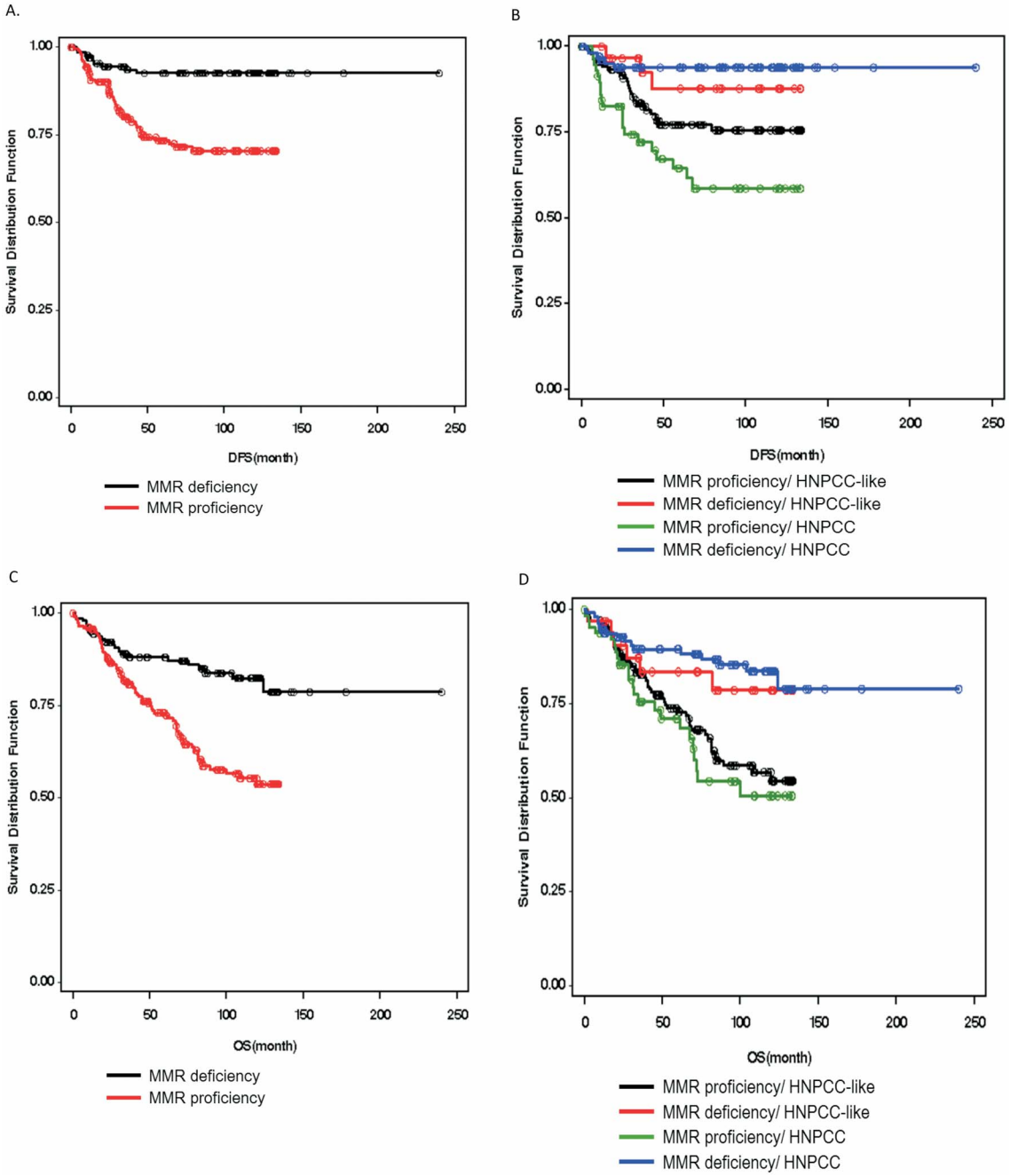


**Fig. 2.** Disease free survival (A and B) and overall survival (C and D) of different subgroups classified using the Amsterdam II criteria and MMR gene expression status. MMR, mismatch repair; HNPCC, hereditary nonpolyposis colorectal cancer.

patients showed significantly worse OS than HNPCC-like patients (HR = 1.991; 95% CI, 1.141-3.464; $p$ = 0.0148; Table 6). However, there were no significant differences between the other subgroups classified by combining MMR gene expression with A-II C (Table 6).

## Discussions

In this study, on the basis of fulfilling AC-II criteria, we included two subgroups, HNPCC versus HNPCC-like, for MMR gene expression analyses. Our study demonstrated 63.3% of clinical cohort ful-

**Table 6.** Comparisons of disease-free survival among subgroups related to Amsterdam-II criteria and MMR gene expression status

| Clinicopathological features | DFS | | OS | |
|---|---|---|---|---|
| | *p*-value* | Adjusted hazard ratio (95% CI) | *p*-value* | Adjusted hazard ratio (95% CI) |
| dMMR vs. pMMR | 0.0132 | 0.329 (0.137-0.792) | 0.0104 | 0.439 (0.234-0.824) |
| HNPCC vs. HNPCC-like | 0.0967 | 1.715 (0.907-3.241) | 0.0148 | 1.991 (1.144-3.464) |
| pMMR/HNPCC-like vs. dMMR/HNPCC-like | 0.3789 | 1.873 (0.463-7.578) | 0.6165 | 1.280 (0.487-3.367) |
| dMMR/HNPCC vs. dMMR/HNPCC-like | 0.9495 | 0.952 (0.208-4.365) | 0.957 | 1.029 (0.364-2.907) |
| pMMR/HNPCC vs. dMMR/HNPCC-like | 0.0779 | 3.527 (0.869-14.324) | 0.0343 | 3.041 (1.086-8.515) |
| TNM_N staging, N2 vs. N0 | < 0.0001 | 5.209 (2.391-11.351) | 0.0014 | 2.838 (1.494-5.390) |
| TNM_N staging, N3 vs. N0 | 0.0036 | 9.229 (2.071-41.119) | 0.0045 | 3.492 (1.474-8.275) |
| TNM_M staging, M1/2 vs. M0 | | | < 0.0001 | 5.317 (3.098-9.125) |
| Age, per 10-year increase | | | 0.0004 | 1.369 (1.151-1.629) |
| Sex, male vs. female | | | 0.0904 | 1.461 (0.942-2.267) |
| Multiple tumors, yes vs. no | | | 0.0305 | 1.746 (1.054-2.893) |

* By multivariate Cox proportion hazard model.
MMR, mismatch repair; HNPCC, hereditary nonpolyposis colorectal cancer; dMMR, mismatch repair deficient; sMMR, mismatch repair sufficient; TNM, tumor node metastasis; CI, confidence interval.

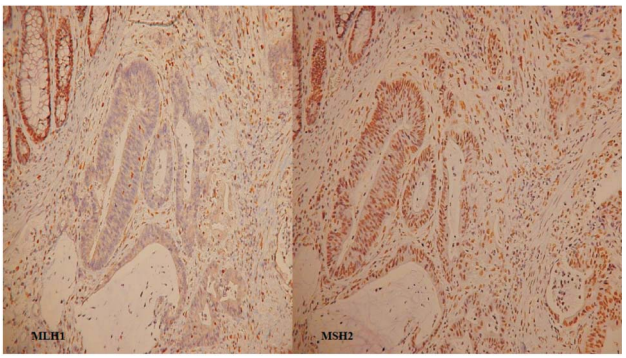


**Fig. 3.** Representative figures of loss of MLH1 staining (left) and intact of MSH2 staining (right). Internal control of lymphocytes showing intact staining of MLH1 or MSH2 proteins.

filling with AC-II proved to be dMMR tumors by MMR testing. However, the patients defined as "HNPCC-like" or "Amsterdam-II minus one criterion", only 16.3 percent proved to be dMMR although the distribution of loss of MMR gene expression did not differ between the HNPCC and HNPCC-like groups (Table 1). As shown in Table 1, the clinicopathologic features of HNPCC-like patients were significantly different from HNPCC generally rather than similar to sporadic CRC patients.

As far as the results presented in this study, some distinct clinicopathological features related to patients with HNPCC or Lynch syndrome were consistent with previous reports including a significantly higher rate of poor differentiation, mucinous/signet ring cell adenocarcinoma, right colon predominant CRC, and a higher risk of developing secondary CRC,[4,7,12] however, we further indicated some novel findings little emphasized before. We first reported that compared to the pMMR tumors, dMMR tumors showed significantly larger tumor sizes, deeper (T4) tumor invasion, less lymph node involvement, and fewer distant metastases (Table 2 and Table 4). The larger tumors might coincide with deeper tumor invasion, despite the fact that these tumors are biologically less aggressive. These findings might further explain improved survival (both DFS and OS) in dMMR patients compared to the pMMR patients (Fig. 2A and 2C), even after adjusting for age, sex, and the TNM staging factors (Table 6). Although the underlying mechanisms remain unclear, immune interactions might affecting the rate of tumor agent-induced escape from immune suppression related to the dMMR status has been observed between the tumor and the host.[13]

Interestingly, in dMMR tumors, significant differences were observed between different strength of family history (HNPCC and HNPCC-like patients) subgroups. In comparison to the dMMR/HNPCC patients, dMMR/HNPCC-like patients showed male predominance and higher rates of poor differentiation

(Table 3). Our results imply if an unknown mechanism affecting the differences in penetrance between the male and female patients, and other mechanisms not related to expressions of MMR genes involved in tumor differentiation during carcinogenesis. Furthermore, significantly higher cumulative incidences were observed (Table 5 and Fig. 1) between the dMMR/HNPCC-like patients group compared with those in the dMMR/HNPCC group. Risk comparisons of the cumulative incidences of patient subgroups showed that the adjusted hazard ratios (HR) for the dMMR/HNPCC-like (10.02; 95% confidence interval (CI), 3.04-33.00; $p < 0.0001$) and dMMR/HNPCC (5.44; 95% CI, 1.62-18.26; $p = 0.006$) groups. This highlighted risk of developing metachronous CRC might be further classified and might help determine extent of colectomy of the HNPCC patients more individually.

We further analyzed the family histories of HNPCC-like patients (patients with "Amsterdam-II minus one criterion") showed that a significantly lower rate of lacking at least one relative diagnosed before the age of 50 was (9.7% vs. 58.6%, $p < 0.001$), and a significantly higher rate of lacking at least three relatives with a Lynch-associated cancer (51.6% vs. 17.9%, $p < 0.001$) was observed in the dMMR/HNPCC-like subgroup compared to pMMR/HNPCC-like subgroup. These findings indicated younger age of diagnosis rather than presence of Lynch-associated cancer for presence of dMMR tumors among HNPCC-like patients. We thus argued that some genes other than MMR responsible for modifying this cohort of patients defined as a group of "Amsterdam-II minus one criterion".

Whether CRC patients with family histories have better survival compared to that of patients with sporadic CRC, remains controversial.[14-17] Recently, Lautrup et al. found that in contrast to the lower mortality in LS patients, survival in other types of familial CRCs does not seem to be affected after diagnosis.[18] These inconsistencies might be resulted from the ratio of the different CRC subgroups included. In this study, patients with LS or dMMR tumors showed better survival than the sporadic ones or patients with pMMR tumors by multivariate analyses (Figs. 2A and 2C, Table 6). However, the patients included in previous studies, with family histories of positive CRC might have different ratios of LS or dMMR that determine the survival benefit. However, these differences could not be further distinguished with family history (HNPCC or HNPCC-like) with same MMR status. Therefore, these findings support that MMR gene expression status is more important than family history that it affects survival, that results might be inconsistent if different ratios of patient subgroups are included.[19,20]

The advantage of this study was that it included a large cohort of patients, with standardized data collection in a single institute, providing the opportunity for in-depth analyses of detailed family histories combined with MMR statuses related to clinicopathological features. Our results still failed to delineate how the expression statuses of MMR genes affected the clinicopathological differences between these patients although our results support that the loss of expression of *MSH6* is rare in HNPCC families.[21,22] Although the loss of *MSH6* has been shown to be associated with older age at onset, the association of the high rate of loss of *MSH6* expression with the development of HNPCC-like disease could not be explained. Furthermore, it should also be noted that even with the multiple statistical comparisons performed in this study, the noted associations could be chance findings.

## Conclusion

On the basis of fulfilling AC-II criteria, we included two subgroups, HNPCC versus HNPCC-like, for MMR gene expression analyses. We reported distinguishing features related to the subgroups of dMMR/HNPCC-like "patients, including male predominance and an extremely high rate of poor differentiation. In addition, risk of developing metachronous CRC might be further classified by combining family history and MMR status.

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## Institutional Review Board Statement

This study was approved by the institutional review board (IRB) of CGMH (IRB102-2284B).

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**原　著**

# 完全符合阿姆斯特丹-II 標準或僅缺少一個標準之結直腸癌病人的臨床病理特徵及錯配修復蛋白表現之差異

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**目的**　遺傳性非息肉性結直腸癌 (HNPCC) 患者被報導具有臨床病理學特徵。本研究比較滿足阿姆斯特丹 II 標準 (A-II C) 或僅缺乏一個標準的患者 (HNPCC-like) 的臨床病理特徵。

**方法**　利用免疫組織化學用於檢測錯配修復 (MMR) 基因表現。Cox 比例風險模型用於研究 A-II C 和 MMR 狀態對生存和臨床病理因素的影響。

**結果**　我們回顧性評估共 380 例大腸直腸癌患者，包括 177 例 HNPCC 患者和 203 例類似 HNPCC 樣病例 (缺乏一種 A-II 標準)。總體而言，63.3% 的 HNPCC 患者和 16.3% 的 HNPCC 樣病例表現出至少一種 MMR 蛋白的喪失。與 MMR-正常 (pMMR）患者相比，MMR 缺陷 (dMMR) 患者具有腫瘤較大 (28 $cm^2$ vs. 18 $cm^2$, $p < 0.0001$)，腫瘤浸潤較深 (T4) (40.7% vs. 29.0%, $p < 0.0173$)，淋巴結轉移率較低 (N0) (31.0% vs. 48.5%, $p = 0.0034$)，及遠處轉移 (M0, 8.3% 對 15.3%, $p = 0.0447$) 較少。與 dMMR / HNPCC 亞組相比，dMMR / 類似 HNPCC 亞組的男性患者 (72.7% vs. 43.8%, $p = 0.0034$) 顯著更多，腫瘤分化差 (42.4% vs. 22.9%, $p < 0.028$) 更高。並且觀察到顯著不同的發展中有限的 CRC 的發生率，從最低 3.28 (pMMR / 類似 HNPCC)，6.18 (pMMR / HNPCC)，20.57 (dMMR / HNPCC) 到最高 37.78 人年 (dMMR / 類似 HNPCC)。

**結論**　我們報告了 dMMR / HNPCC 樣患者亞組相關的顯著特徵，包括男性佔優勢，極低分化率和發生異時 CRC 的風險。因此，經由結合家族史和 MMR 狀態，可以進一步對發生異時性結直腸癌的風險進行分類。

**關鍵詞**　阿姆斯特丹-II 標準、錯配修復蛋白表現缺陷、異時性結直腸癌、分化不良、男性。