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Original Article

Frequency and clinical features of deficient mismatch repair in ovarian clear cell and endometrioid carcinoma

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ABSTRACT

Objective: To clarify the frequency of deficient mismatch repair (dMMR) in Japanese ovarian cancer patients, we examined microsatellite instability (MSI) status and immunohistochemistry (IHC) subtypes, including endometrioid carcinoma (EMC), clear cell carcinoma (CCC), or a mixture of both (Mix).
Methods: We registered 390 patients who were diagnosed with EMC/CCC/Mix between 2006 and 2015 and treated at seven participating facilities. For 339 patients confirmed eligible by the Central Pathological Review Board, MSI, IHC, and MutL homolog 1 methylation analyses were conducted. The tissues of patients with Lynch syndrome (LS)-related cancer histories, such as colorectal and endometrial cancer, were also investigated.
Results: MSI-high (MSI-H) status was observed in 2/217 CCC (0.9%), 10/115 EMC (8.7%), and 1/4 Mix (25%). Additionally, loss of MMR protein expression (LoE-MMR) was observed in 5/219 (2.3%), 16/115 (14.0%), and 1/4 (25%) patients with CCC, EMC, and Mix, respectively. Both MSI-H and LoE-MMR were found significantly more often in EMC ($p<0.001$). The median (range) ages of patients with MMR expression and LoE-MMR were 54 (30–90) and 46 (22–76) ($p=0.002$), respectively. In the multivariate analysis, advanced stage and histological type were identified as prognostic factors.
Conclusion: The dMMR rate for EMC/CCC was similar to that reported in Western countries. In Japan, it is assumed that the dMMR frequency is higher because of the increased proportion of CCC.

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Presentation

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Conflict of Interest

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published. No potential conflict of interest relevant to this article was reported.

Author Contributions

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Keywords: Ovarian Cancer; Microsatellite Instability; Adenocarcinoma, Clear Cell; Endometrioid; Lynch Syndrome

Synopsis

The deficient mismatch repair (dMMR) rate in Japanese patients is currently unknown. This study determined the frequency of dMMR in Japanese ovarian cancer patients. The dMMR rate was similar to that reported in Western countries. We identified potential criteria for implementing microsatellite instability and immunohistochemistry analyses in Lynch syndrome screening.

INTRODUCTION

Approximately 10%–15% of ovarian cancers are hereditary. The most frequent and notable type is hereditary breast and ovarian cancer (HBOC) caused by pathogenic variants in *BRCA1/2*, followed by Lynch syndrome (LS) due to congenital abnormalities in mismatch repair (*MMR*) genes accounting for 10% [1-4].

The MMR pathway repairs base pair mismatches that originate during DNA replication, thereby maintaining a high degree of genomic sequence integrity. *MMR* gene variants and epigenetic changes, such as *MMR* gene promoter methylation, can disrupt this pathway and result in a deficient MMR (dMMR) response. This may lead to the development of cancer because of the accumulation of mutations in the genome, which are more likely to occur in microsatellite regions. The presence or absence of dMMR can be investigated by determining the microsatellite instability-high (MSI-H) status in tumor tissues. Immunohistochemistry (IHC) of the MMR proteins MutL homolog 1 (MLH1), MSH2, MSH6, and PMS1 homolog 2, mismatch repair system component (PMS2) in tumor tissues can also be performed to assess MMR protein function and analyze the presence of dMMR.

HBOC is observed more frequently with high-grade serous carcinoma, which is the most common histological type of ovarian cancer. dMMR is more commonly observed in endometrioid carcinoma (EMC) and clear cell carcinoma (CCC) cases [5-10]. Although the frequency of *BRCA1/2* mutation in ovarian cancer has been revealed in recent years, the incidence of dMMR in Japanese patients remains unknown. Because ovarian CCC is refractory to chemotherapy, the development of new treatment strategies is an urgent issue, and immune checkpoint inhibitors have recently been shown to be effective in solid tumors with dMMR status. Hence, elucidating the frequency and clinical features is of great significance to optimize the therapeutic strategy.

Here, we examined the dMMR frequency in Japan by performing IHC of MMR proteins and MSI tests in ovarian cancer cases pathologically diagnosed as EMC, CCC, or a mixture of both subtypes (Mix). We also analyzed the association between MMR status and clinical features, including the presence or absence of *MLH1* methylation.

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MATERIALS AND METHODS

Three-hundred and ninety patients treated for ovarian cancer at seven participating facilities and diagnosed with EMC/CCC/Mix according to the 2003 World Health Organization classification by postoperative pathology from 2006–2015 were registered. The age, clinical stage based on the 2014 International Federation of Gynecology and Obstetrics classification, treatment start date, outcome, medical history, and family history were extracted from the medical records of 339 patients considered pathologically eligible by the Central Pathological Review Board (CPR) (**Fig. S1**). MSI and IHC analyses were conducted in 339 eligible patients. The tissues of patients with LS-related cancer complications or histories were also investigated.

The CPR examined the validity of pathological diagnosis and MMR protein expression in IHC analyses. Each sample was microscopically examined by two pathologists. If their observations matched, the diagnosis was confirmed. If the results were inconsistent, another pathologist examined the sample and made the final diagnosis. For MSI testing, the percentage of tumor cells in the specimens was assessed by the CPR to select the optimal site.

This study was conducted with the approval of the National Hospital Organization Clinical Research Central Ethics Review Committee and the permission of the directors of the seven participating facilities. Informed consent was given in the form of opt-out in accordance with Japanese ethical guidelines.

1. IHC

Unstained tissues with a 4–5-μm thickness were collected from each institution. Formalin-fixed and paraffin-embedded tissues were sectioned and protected with a tissue protector (Matsunami Glass Ind. Ltd. Osaka, Japan). Antigen activation was performed using CC1 buffer for 64 minutes at 95°C and pH 9.0. MMR protein expression was analyzed using a VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody, VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody, VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody, and VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody from Roche Diagnostics (Tucson, AZ, USA), and all antibodies were pre-diluted.

Tissues were counterstained with hematoxylin using the Ventana ultraView DAB Universal Kit (ROC-109431) and BenchMark ULTRA (Ventana Medical Systems, Tucson, AZ, USA). Nuclear staining of normal lymphocytes and interstitial cells served as a positive control. Only nuclear staining was accepted as positive protein expression.

2. Microsatellite Analysis

DNA was extracted using a QIAamp DNA FFPE Tissue Kit (Qiagen, Duesseldorf, Germany) and denatured following the manufacturer's instructions. Five mononucleotide markers, including BAT25, BAT26, NR-21, NR-24, and MONO-27 (MSI Analysis System v 1.2; Promega, Charbonnieres-les-Bains, France), were detected by a multiplex polymerase chain reaction (PCR). Because the quasi-monomorphic variation range of these markers fell within a certain range regardless of race [11], fragment analysis was performed at the tumor site only. MSI-H was determined when abnormalities in the number of repetitions were observed in two or more regions. MSI-low (MSI-L) was determined when only one region was abnormal, and microsatellite stable (MSS) was determined when no abnormalities were observed.

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3. Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA)

In cases with decreased MLH-1 protein expression, methylation analysis of CpG islands in the *MLH1* region was performed with the MS-MLPA method using the SALSA MS-MLPA ME011-B3 MMR Genes Kit (MRC-Holland, Amsterdam, The Netherlands) following the manufacturer's instructions. This kit contains 5 probe regions (**Table S1**) for the *MLH1* gene.

Extracted DNA samples were heat-denatured and hybridized with the SALSA probemix. The specimens were divided into two parts: one was digested with the methylation-sensitive restriction enzyme HhaI and ligated, and the other was ligated without HhaI (30 minutes, 48°C). After inactivating the ligase and HhaI enzymes, polymerase was added for PCR. For PCR fragment analysis, capillary electrophoresis was performed using an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Finally, the detected amplification peaks were compared between samples with and without HhaI, and the methylation percentage was calculated using Coffalyser.NET (MRC-Holland). To calculate the methylation percentage, the fluorescence value of the signal for each probe was divided by the signal of each reference probe. The median value of all ratios for each probe was obtained and used as the normalizing constant of the probe. Furthermore, the methylation percentage was obtained by dividing the normalizing constant of the digested sample by the normalizing constant of the undigested sample of the same probe. Based on previous studies [12], the threshold was set to 20% for probes 3 and 4 to determine the presence or absence of methylation.

4. Data analysis

Data analysis was performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA). Fisher's exact test was used to determine the association between IHC and MSI results for each histological type. Concordance between IHC and MSI results was assessed using the κ coefficient. Any differences in the percentage of MSI-H by the type of protein with reduced expression were tested using Fisher's exact test. Analysis of the clinical features in loss of MMR expression (LoE-MMR) and MMR expression groups was conducted using the Wilcoxon rank-sum test for age and Fisher's exact test for other factors. The hazard risk (HR) was then estimated by univariate and multivariate analyses using a Cox proportional hazards model. Survival analysis was performed with the Kaplan–Meier method. We also analyzed the clinical features and MMR status in cases with a history of multiple LS-related cancers listed in the Bethesda guidelines including endometrial cancer, colorectal cancer (CRC), small intestine cancer, and gastric cancer. The level of statistical significance was set at $p<0.05$.

RESULTS

Among the 339 subjects, there were 219 (65%), 116 (34%), and 4 (1%) patients with CCC, EMC, and Mix, and their median (range) ages were 54 (34–90), 51 (22–85), and 54 (22–90) years, respectively. The percentages of clinical stage I/II cases were as follows: all=81% (274/339), CCC=82% (180/219), EMC=78% (91/116), and Mix=75% (3/4) (**Table 1**).

MSI status was unanalyzable in 3/339 patients (0.88%). Therefore, we analyzed 217, 115, and 4 patients with CCC, EMC, and Mix, respectively. Two CCC (0.9%), 10 EMC (8.7%), and 1 Mix (25%) patient were classified as MSI-H. MSI-H was significantly more frequent in EMC cases than in CCC, except for Mix cases (**Table 2**) (Fisher's exact test, $p<0.001$).

Table 1. Clinical characteristics

Characteristics	CCC	EMC	Mix	Total
Total No. of patients	219	116	4	339
Age (yr)	54 (34–90)	51 (22–85)	54 (22–90)	54 (22–90)
Stage (FIGO 2014)				
I–II	180	91	3	274
III–IV	39	25	1	65
Value of CA125	61 (3–39,350)	142 (5.6–18,850)	904 (318.8–2,207.1)	100.6 (3–39,350)
Neoadjuvant chemotherapy				
Yes	5	11	0	16
No	214	105	4	323

Values are presented as frequency or median (range).
CA125, cancer antigen 125; CCC, clear cell carcinoma; EMC, endometrioid carcinoma; FIGO, International Federation of Gynecology and Obstetrics; Mix, mixed subtype.

Table 2. MSI and IHC results for each ovarian cancer histological subtype

Analysis	CCC	EMC	Mix	Total
MSI				
MSI-H	2/217 (0.9%)*	10/115 (8.7%)*	1/4 (25%)	13/336 (3.9%)
MSI-L/MSS	215/217 (99.1%)	105/115 (91.3%)	3/4 (75%)	324/336 (96.1%)
IHC				
LoE-MMR	5/219 (2.3%)†	16/115 (14.0%)†	1/4 (25%)	22/338 (6.6%)
MLH1, PMS2‡	0/219 (0%)	5/115 (4.3%)	0/4 (0%)	5/338 (1.5%)
MSH2, MSH6	4/219 (1.8%)	8/115 (7.0%)	1/4 (25%)	13/338 (3.9%)
MSH6	1/219 (0.5%)	2/115 (1.8%)	0/4 (0%)	3/338 (0.9%)
PMS2	0/219 (0%)	1/115 (0.9%)	0/4 (0%)	1/338 (0.3%)
E-MMR	214/219 (97.7%)	99/115 (86.0%)	3/4 (75%)	316/338 (93.4%)

CCC, clear cell carcinoma; EMC, endometrioid carcinoma; E-MMR, expression of mismatch repair protein; IHC, immunohistochemistry; LoE-MMR, loss of mismatch repair protein expression; Mix, mixed subtype; MLH1, MutL homolog 1; MSH2, MutS homolog 2; MSH6, MutS homolog 6; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite stable; PMS2, PMS1 homolog 2.
Significant p-value for the combination of EMC and CCC was shown for MSI-H (*$p<0.001$) and LoE-MMR (†$p<0.001$). ‡One out of 5 cases with loss of MLH1 or PMS2 expression showed methylation in the *MLH1* promotor region by methylation-specific multiplex ligation-dependent probe amplification.

For IHC, 1/339 patients (0.29%) could not be tested. Therefore, 219, 115, and 4 patients with CCC, EMC, and Mix, respectively, were assessed. The proteins with the greatest loss of expression were MSH2/MSH6, which was observed in 4 CCC, 8 EMC, and 1 Mix patient. LoE-MMR was found significantly more in EMC than in CCC ($p<0.001$) (**Table 2**). *MLH1* methylation was observed in 1/5 patients (20%) with decreased MLH1/PMS2 expression.

We determined the relationship between LoE-MMR and MSI-H in 335 subjects available for testing (**Table S2**). Among patients with LoE-MMR, the numbers of MSI-H cases were as follows: MLH1/PMS2, 4/5 (80%); MSH2/MSH6, 8/13 (62%); MSH6, 0/3 (0%); and PMS2, 1/1 (100%). The status of 313 patients who did not show decreased expression was as follows: MSI-L=8 patients, MSS=305, and MSI-H=none. MSH6 showed MSS in all 3 cases. Concordance between LoE-MMR/E-MMR and MSI-H/MSI-L/MSS was substantial at $\kappa=0.73$ (95% confidence interval=0.56–0.90) (Landis & Koch criteria). Conversely, no significant differences were observed in the percentage of MSI-H by the type of protein with lost expression ($p=0.140$).

The median ages of patients with E-MMR and LoE-MMR were 54 (30–90) and 46 (22–76) years, respectively, with a tendency of LoE-MMR ($p=0.002$) to be observed in significantly younger patients (**Table 3**). When patients were divided based on age (less than 50 years and older than 50 years), LoE-MMR was observed significantly more in the less than 50 years ($p<0.001$) group. Regarding histology, LoE-MMR was significantly more common in

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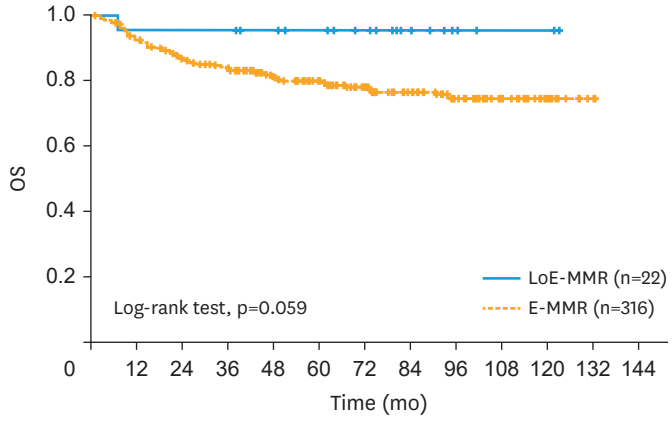
Table 3. Characteristics of the patients with LoE-MMR

Characteristics	E-MMR (n=316)	LoE-MMR(n=22)	p-value
Age (yr)	54 (30–90)	46 (22–76)	0.002
>50	102	15	<0.001
≤50	214	7	
Stage (FIGO 2014)			
I/II	254	19	
III/IV	62	3	0.779
Histological type			
EMC*	99	16	<0.001*
CCC*	214	5	(EMC, CCC)
Mix	3	1	
Double cancer			
+	21	7	<0.001
–	295	15	
Family history			
+	31	4	0.265
–	285	18	

Values are presented as frequency or median (range).
CCC, clear cell carcinoma; EMC, endometrioid carcinoma; E-MMR, expression of mismatch repair protein; FIGO, International Federation of Gynecology and Obstetrics; LoE-MMR, loss of mismatch repair protein expression; Mix, mixed subtype.
*Regarding histology, LoE-MMR was significantly more frequent in EMC compared with the CCC cases (p<0.001).

EMC cases compared with CCC cases (p<0.001). No significant differences were observed in patients with the presence or absence of a family history of LS-related cancers (p=0.265); however, the presence of multiple LS-related cancers was significantly higher in the LoE-MMR group (p<0.001).

Univariate analysis of prognostic factors showed that overall survival (OS) was statistically significant with respect to advanced stage (stage III/IV) (HR=9.03, p<0.001) and CCC (HR=2.04, p=0.015) (**Table S3**, **Fig. S2**). In the multivariate analysis, advanced stage and histological type were identified as prognostic factors (**Table S4**). We did not identify a statistically significant association between MMR status and prognosis, but dMMR tended to be a good prognostic factor (p=0.059; **Fig. 1**).



No. of patients at risk													
LoE-MMR	22	21			18	16	13	8	4	2		0	
E-MMR	316	287	258	240	189	161	123	96	62	38	21	2	0

Fig. 1. Analysis of survival regarding MMR status.
E-MMR, expression of mismatch repair protein; LoE-MMR, loss of mismatch repair protein expression; OS, overall survival.

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We next focused on 24 patients with a history of multiple LS-related cancers. The median (range) age was 49 (30–71) years, and the histological subtypes were as follows: EMC=20 (83%), CCC=3 (13%), and MC=1 (4%). Clinical stages I/II were observed in 20 cases (83%), and stages III/IV were observed in 4 cases (17%). Regarding the multiple primary cancers related to LS, there were 21 (88%), 1 (4%), and 2 (8%) patients with endometrial cancer, CRC, and endometrial/CRC, respectively (**Table 4**). LoE-MMR was observed in 7/24 cases (29%), including 2 CCC and 5 EMC patients. The median (range) age was 41 (30–50) years. A previous history of endometrial cancer was found in all patients, and their histological types were all EMC. Two also had a history of CRC. Family history was present in 5 cases (21%). The proteins showing loss of expression were as follows: MSH2/MSH6 in 3 (43%), MLH1/PMS2 in 3 (43%), and MSH6 in 1 (14%). MSI-H was observed in 4 cases (57%). When comparing the test results between ovarian cancer tissues and other cancer regions in patients with multiple LS-related cancers and dMMR, which are thought to be associated with increased LS characteristics, the same protein showed loss of expression in both tissues in 57% (4/7) of patients, and the same MSI status was observed in 71% (5/7) (**Table S5**).

DISCUSSION

In Western countries [5-10], dMMR has been reported in 0.3% of serous carcinoma (SC) cases, 12.5% of EMC cases, and 3.0% of CCC cases (**Table 5**). In this study, dMMR was

Table 4. Characteristics of patients with multiple cancers

Characteristics	Values
Age (yr)	49 (30–71)
Histological type (ovarian cancer)	
CCC	20 (83%)
EMC	3 (13%)
Mix	1 (4%)
Clinical stage (FIGO 2014)	
I	14 (58%)
II	6 (25%)
III	4 (17%)
IV	0
LS-associated tumor	
Endometrial cancer	21 (88%)
Colorectal cancer	1 (4%)
Endometrial and colorectal cancer	2 (8%)
Family history of LS-associated tumor	
Yes	5 (21%)
No	19 (79%)

CCC, clear cell carcinoma; EMC, endometrioid carcinoma; FIGO, International Federation of Gynecology and Obstetrics; LS, Lynch syndrome; Mix, mixed subtype.

Table 5. Frequency of dMMR in ovarian cancer cases

Study	Year	No.	Results					
			SEC	EMC	CCC	MUC	Mix	Other
Malander et al. [5]	2006	128	0/84 (0%)	0/15 (0%)	1/22 (4.5%)	1/5 (20%)	1/2 (50%)	
Rosen et al. [6]	2006	322	0/168 (0%)	1/34 (2.9%)	1/16 (6.2%)	0/7 (0%)	3/73 (4.1%)	1/31 (3.2%)
Jensen et al. [7]	2008	52	0/19 (0%)	3/18 (16.7%)	1/8 (12.5%)		0/4 (0%)	0/3 (0%)
Lu et al. [8]	2012	290	2/182 (1.1%)	2/29 (6.9%)	1/27 (3.7%)	0/7 (0%)	3/5 (60%)	1/40 (2.5%)
Rambau et al. [9]	2016	612	0/149 (0%)	25/181 (13.8%)	4/163 (2.4%)	0/93 (0%)		0/26 (0%)
Fraune et al. [10]	2020	478	1/358 (0.3%)	8/35 (23%)	0/23 (0%)	0/34 (0%)	0/11 (0%)	0/17 (0%)
Total		1,882	3/960 (0.3%)	39/312 (12.5%)	8/259 (3.0%)	1/136 (0.7%)	7/95 (7.3%)	2/117 (1.7%)

CCC, clear cell carcinoma; dMMR, deficient mismatch repair; EMC, endometrioid carcinoma; Mix, mixed subtype; MUC, mucinous carcinoma; SEC, serous carcinoma.

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observed in 16/114 (14.0%) EMC and 5/218 (2.3%) CCC cases. Hence, it was significantly more common in EMC cases, similar to the reports in Western countries.

The incidence of epithelial ovarian malignancies in Western countries is approximately 10% for both EMC and CCC and 70% for SC [13]. In Japan, according to the Gynecologic Oncology Registry of the Japan Society of Obstetrics and Gynecology, the incidence of SC from 2016–2018 was 40.3%–43.2%, followed by 23.8%–24.8% for CCC and 16.8%–17.2% for EMC, indicating a higher percentage of EMC/CCC. Based on the findings of this study, the dMMR rate in Japanese ovarian cancer patients was higher than that observed in Western countries [13].

In this study, CpG island hypermethylation responsible for dMMR was also assessed. Hypermethylation is often used to discriminate between sporadic and inherited types. However, it is not possible to accurately distinguish between these types because *MLH1* germline mutation and *MLH1* promoter hypermethylation might coexist [14], and there is an assumption that *MLH1* promoter hypermethylation is the "second hit" in hereditary cases [15]. Studies of clinical features, including methylation status and outcomes, are increasing, mainly in the CRC field [16]. In gynecological cancers, the frequency of methylation ranged from 71%–97% in uterine corpus cancer patients with *MLH1* mutation [17-19] and 80%–100% in EMCs [10,20]. However, the number of published studies is limited. In our study, *MLH1* promoter hypermethylation was observed in only 1/5 (20%) patients with suspected loss of *MLH1* gene expression. Hence, it is difficult to analyze the methylation phenotype. However, we hope that the accumulation of more evidence globally will help elucidate the relationship with clinical features in ovarian cancer.

The comparison of test results between ovarian cancer tissues and other cancer regions in patients with multiple LS-related cancers revealed that both sites showed the same IHC and MSI status in more than half of the cases, and dMMR was speculated to be involved in the carcinogenic mechanism in both tissues. For case 4 (**Table S5**), MLH1/PMS2 were reduced in ovarian cancer tissues, and MLH1/PMS2/MSH6 were decreased in endometrial cancer tissues. In addition to *MLH1* gene mutation, *MSH6* gene mutation was identified in endometrial cancer. This can be attributed to the hypothesis that some *MMR* genes have repetitive sequences [21] and undergo secondary mutations because of disrupted MMR by the original mutation. Although MSH6 expression disappears in tumor tissues after chemotherapy [22], this case did not have a history of treatment before surgery.

The prognostic effect of the MMR status has also been examined in several carcinomas. For example, early-stage CRC cases with dMMR showed a better prognosis compared with proficient MMR (pMMR) cases [23]. However, a negative prognostic effect was observed in dMMR cases with BRAF V600E mutation (i.e., cases with *MLH1* promoter hypermethylation). Additionally, MSI-H is a negative prognostic factor in recurrent and metastatic CRC cases, unlike in early-stage patients. In endometrial cancer, many studies have shown a negative prognostic effect, although there was no statistically significant association between MSI-H and OS [24]. This was partly attributed to the limited literature because of differences in the testing and selection of histological types.

In ovarian cancer, no differences were observed in the 5-year OS between LS-associated ovarian cancer (LSAOC) and non-LSAOC cases [25,26], and there was no difference due to MMR status in the advanced stage. However, progression-free survival was prolonged in cases with dMMR compared with pMMR in the early stage [27]. To date, no consensus has been established. In

our study, a statistically significant association was not observed between the MMR status and survival rate. This might be attributed to the small number of events to assess OS in the dMMR group. The prognostic impact of dMMR may be dependent on stages, initial/recurrent disease, somatic/germline differences [23-27]. Hence, studies with a larger sample size and various conditions are required. MSI tests performed as a companion diagnostic assay of immune checkpoint inhibitors may help clarify the clinical features associated with MMR status.

LS is the second most commonly observed familial ovarian tumor after HBOC. Its diagnosis contributes to the surveillance of metachronous cancer in patients and the health management of family members. High-risk patients are clinically identified by primary screening using the Amsterdam Criteria II and revised Bethesda guidelines, which account for the age of onset, family history, and history of LS-related tumors. They then undergo secondary screening with MSI tests and IHC analysis of MMR proteins, followed by genetic testing if any findings are abnormal. However, ovarian cancer is not included in the Amsterdam Criteria II for LS-related tumors. Furthermore, the revised Bethesda guidelines are difficult to apply in carcinomas other than CRC as the guidelines generally apply to CRC. Additionally, screening with these clinical parameters is not very sensitive, with 12%–28% of LS cases overlooked [28,29]. Therefore, in Western countries, it is recommended that the primary screening be omitted in patients with colorectal or endometrial cancer and that secondary screening with MSI or IHC testing is conducted for all patients. However, the cost-effectiveness of screening for other carcinomas has not yet been demonstrated. Regarding ovarian cancer, primary screening for high-risk patients followed by MSI testing/IHC analysis of MMR proteins will be a useful strategy. Our findings indicated that only 18% of LoE-MMR cases had a family history of LS-related cancers, and MMR status was associated with the presence of multiple LS-related cancers rather than family history. At least 3 familial cases of LS-related cancer are required in the Amsterdam Criteria II, and criteria related to family history account for 2/5 in the revised Bethesda guidelines. However, our results suggest that the presence of multiple LS-related cancers, rather than family history, should be considered when analyzing LS in ovarian cancer cases. Based on our findings, patients with EMC/CCC histological types, especially those with younger age (<50 years) or concurrent/previous multiple LS-related cancers, may be good targets for IHC or MSI testing during LS screening.

In this study, IHC and MSI testing showed that 13/22 patients (59%) were LoE-MMR. All 316 patients with MMR expression were classified as MSI-L/MSS. The concordance between the 2 tests was 97.3%, indicating statistical significance. Good concordance of 96%–97% has also been reported for CRC [30,31]. However, Lee et al. reported a value of only 67.6% in ovarian cancer compared with other carcinomas [32]. In their study, the percentages of LoE-MMR and MSI-H in all patients were 27% and 15%, respectively, and the false positive rate of LoE-MMR was higher than that reported in previous studies. This discrepancy may be attributed to the different IHC staining methods (manual or automated), subjectivity of judgment, and storing of tumor tissues. In our study, IHC analysis by two experts improved the objectivity. Additionally, the increased sensitivity of MSI markers used in this study contributed to a higher concordance rate. The use of mononucleotide markers instead of NCI markers for MSI determination has increased. They are more suitable for clinical practice because of their high sensitivity and convenience of requiring tumor tissue only. It has been shown that tumors displaying dMMR are more responsive to immune checkpoint inhibitors than pMMR tumors [33]. Additionally, the MMR status in 60% of patients with MSI-H or LoE-MMR by local assessment and primary resistance to immune checkpoint inhibitors was reported to be inaccurate after reassessment [34], which highlights the importance of examination accuracy.

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In this study, all cases with LoE of MSH6 showed MSS, and the concordance rate tended to be lower. The function of MSH6 is complemented by other proteins, such as MSH3, even if MSH6 is deleted [35,36]. This might be a limitation of the MSI test. Shia et al. reported that 75% of patients with reduced MSH6 expression were not classified as MSI-H [37]. Our study also demonstrated similar outcomes.

Using a central pathology diagnosis to confirm pathological eligibility is one of the strengths of our study. Additionally, the MSI markers used in this study reflect real-world settings because they are used for companion diagnostics in clinical practice.

Furthermore, the target patients in this study were limited to those who underwent surgery and were pathologically diagnosed. Selection bias may have existed because this is a case-control study. In our multi-institutional study, there were no uniform protocols between the centers for the preservation of surgically removed tissues, and some included samples were stored for longer than 10 years. Although the storage period might affect DNA quality indicators, such as the PCR yield and Q value, both IHC and MSI assays were performed with a high probability of 99.7% and 99.1%, respectively. However, the quality of sampling to tissue preparation is a topic for future study because it is directly related to the accuracy of IHC or MSI testing. As the clinical application of genome analysis progresses, the information obtained is directly linked to the treatment policy, and accuracy is required. In the future, it is important to standardize tissue preservation methods.

In this study, we examined the frequency of LoE-MMR and MSI-H in EMC and CCC cases. The dMMR rate for each tissue type was similar to that reported in Western countries. Therefore, we speculate that the dMMR rate in ovarian cancer is higher in Japan, where the percentage of EMC/CCC histological types is higher than that observed in Western countries. The implementation of LS screening would lead to early diagnosis or prevention of LS-related cancers for individuals with LS. Although there is no evidence to support the universal screening of LS in ovarian cancer, IHC or MSI testing should be performed in EMC/CCC ovarian cancer patients, especially those with younger age (<50 years) or multiple LS-related cancers.

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SUPPLEMENTARY MATERIALS

Table S1

Targeted probe of *MMR* genes for methylation analysis

Click here to view

Table S2

Relationships between IHC and MSI results

Click here to view

Table S3
Univariate analysis of prognostic factors

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Table S4
Multivariate analysis of prognostic factors

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Table S5
dMMR in patients with multiple cancers

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Fig. S1
Patient enrollment in this study.

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Fig. S2
Analysis of survival regarding histological type.

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