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ORIGINAL ARTICLE

Clinicopathological features of breast cancer in Japanese female patients with Lynch syndrome

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Abstract

Background Lynch syndrome (LS) is a predominantly inherited syndrome caused by a pathological germline mutation in one of the mismatch repair (MMR) genes. Whether breast cancer (BC) is one of the LS-associated tumors is controversial. The aim of this retrospective cohort study was to evaluate the clinical features of BC in Japanese patients with LS.

Methods Of 38 mutation carriers, 4 females with BC were examined in this study.

Results Two of the four patients had multiple BC. Their median age at the diagnosis of BC was 63 (range, 47–84) years. The TNM (6th revision) stages of the six BCs were as follows: stage I, 33% (2/6); stage IIA, 50% (3/6); and stage IIB, 17% (1/6). Histological examination revealed four scirrhous, one papillotubular, and one medullary carcinoma. The positive ratios for estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth receptor 2 (HER2) were 83.3% (5/6), 83.3% (5/6), and 16.7% (1/6), respectively. Two of the three specimens showed MSI-H and one showed MSS. These MSI-H BCs had tumor-infiltrating lymphocytes. Two of the three specimens showed an absence of MLH1 and PMS2 proteins on immunohistochemistry. The cumulative risks for a person with LS to develop BC were 4.35% at the age of 50 years, 8.70% at 60 years, and 21.5% at 70 years.

Conclusions Our study results showed BC in Japanese females with LS to be an MSI-H tumor, which was ER and PgR positive and HER2 negative.

Keywords Breast cancer · Lynch syndrome · Mismatch repair gene

Introduction

Lynch syndrome (LS) is a predominantly inherited syndrome caused by a pathological germline mutation in one of the mismatch repair (MMR) genes [1]. It is the most common syndrome for inherited colorectal cancer, responsible for approximately 2–4% of all colorectal cancers [1]. Carriers of these mutations also have an increased lifetime risk of developing extracolonic tumors, such as endometrial, gastric, ovarian, pancreatic, ureteral, renal pelvic, bile duct, and brain (usually glioblastoma) tumors. The syndrome may also include sebaceous gland adenomas, keratoacanthomas, and carcinomas of the small bowel, although these occur at a somewhat lower incidence than do other tumors [2]. A few studies have suggested that in Western countries, breast cancer (BC) may be one of the LS-associated tumors; however, whether this is the case remains controversial [3]. There have been few studies in East Asia on association of BC with LS.

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The aim of this retrospective cohort study was to evaluate the clinical features of BC in Japanese patients with LS.

Materials and methods

Families, samples, and data collection

This retrospective, single-institution study of LS patients in Japan included patients who visited Iwakuni Clinical Center between January 1978 and November 2015. Patients underwent genetic testing if they met the revised Bethesda guidelines [2] or a modified version of the Amsterdam II Criteria [4], which included gastric cancer as one of the LS-associated tumors, as this is common in Asian patients with LS. Data were collected for the germline mutation carriers and obligate carriers. The tumor spectrum and clinicopathological characteristics of BCs in LS families were analyzed.

Microsatellite instability analysis

Microsatellite instability (MSI) testing was performed on paraffin-embedded tumors resected during biopsy or operation. MSI testing was performed using five markers (D2S123, D5S346, D17S250, BAT25, and BAT26) recommended by the National Cancer Institute [5]. MSI was rated as high frequency (MSI-H) if two or more markers showed instability or low frequency (MSI-L) if a single marker showed instability.

Immunohistochemistry

Immunohistochemistry (IHC) of BCs in LS was examined by testing levels of estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth receptor 2 (HER2), and MMR proteins (MLH1, MSH2, MSH6, and PMS2). The analysis was performed using antibodies against ER protein (SP1, Roche), PgR (1E2, Roche), HER2 (4B5, Roche), MLH1 protein (clone ES05, Dako), MSH2 protein (FE11, Dako), MSH6 protein (EPR3945, Abcam), and PMS2 protein (A16-4, Biocare Medical). All cases were independently evaluated by a pathologist.

Germline mutation analysis

Genomic DNA was extracted from peripheral blood samples using the standard phenol extraction/purification procedure. Germline mutation analyses were performed with the direct sequencing of the entire coding region in the *MLH1, MSH2*, and *MSH6* genes [6]. If there were no deleterious mutations in the genes, multiplex ligation-dependent probe amplification was performed using a SALSA MLPA *MLH1*/*MSH2* probemix assay. When a pathogenic mutation of the MMR gene was detected, the patient was diagnosed with LS. Prior to the genetic testing, the patients received genetic counseling from clinical geneticists, and they provided written informed consent. The study was approved by the Institutional Review Board of Iwakuni Clinical Center.

Statistical analysis

The cumulative risk of developing BC for a female in LS was analyzed using a Kaplan–Meier plot. Data are presented as totals, medians (range), or percentages. All analyses were performed using SPSS for Windows software program (version 22; SPSS, Inc., Chicago, IL, USA).

Results

Mutations identified in the *MLH1* and *MSH2* genes and tumor spectrum

We identified 25 Japanese LS families wherein the probands had LS-associated tumors such as colorectal, small intestine, biliary, endometrial, and ovarian cancers. 16 families were found to have a mutation of *MLH1* and 9 of *MSH2*. Among the female family members, there were 21 and 8 carriers, with 9 and 0 obligate mutation carriers, of *MLH1* and *MSH2* mutations, respectively. Written informed consent was obtained from these 38 females. Among these 38 females with LS, the tumor spectrum showed 35 colorectal cancers, 17 uterine cancers, 7 gastric cancers, 6 BCs, and 10 other tumors (Table 1).

Clinical features of BC in the LS patients

Among the 38 females, 4 had BC, of which 2 had multiple contralateral BC (Table 2). The median age of these patients at the diagnosis of BC was 63 (range, 47–84) years. Notably, no patient developed BC as an initial cancer. The TNM (6th revision) stages of the six BCs were as follows: stage I, 33% (2/6); stage IIA, 50% (3/6); and stage IIB, 7% (1/6). Locations of the BCs were 67% (4/6) in the left and 33% (2/6) in the right breast.

Table 1 The tumor spectrum for 38 females with Lynch syndrome

Tumor site	Number of cancer lesions
Colorectal	35
Uterus	17
Gastric	7
Breast	6
Others	10
Total	75

Table 2 Medical history of cases of breast cancer in females with Lynch syndrome

Case	MMR gene mutation	Mutation status	Bethesda	Amsterdam II	Age	Tumor site
1	*MLH1*	Mutation carrier	+	+	30	Colorectal (T)
					47	Breast
					48	Colorectal (A)
2	*MLH1*	Mutation carrier	+	+	34	Colorectal (C)
					60	Gastric
					69	Endometrial
					70	Breast
					78	Colorectal (R)
					84	Breast
3	*MSH2*	Mutation carrier	+	–	21	Colorectal (C)
					32	Colorectal (D)
					40	Colorectal (T)
					51	Breast
4	*MSH2*	Mutation carrier	+	–	46	Colorectal (A)
					48	Small intestine
					57	Colorectal (S)
					63	Bilateral breast
					68	Colorectal (S)
					68	Small intestine

C cecum, *A* ascending, *T* transverse, *D* descending, *R* rectum

Histological examination demonstrated four scirrhous carcinomas, one papillotubular carcinoma, and one medullary carcinoma (Table 3). The positive ratios for ER, PgR, and HER2 were 83.3% (5/6), 83.3% (5/6), and 16.7% (1/6), respectively. Further, three of the six BCs were not available for hematoxylin and eosin (H&E) staining and MSI testing because the patients of cases 3 and 4 underwent operation at other hospitals; therefore, we could not obtain their tissue samples. Two of these three specimens had rich tumor-infiltrating lymphocytes (TILs) for H&E staining (Supplementary Fig. 1). These two specimens showed MSI-H and another specimen with poor TILs showed MSS. Three of the four BCs were available for IHC; two of the three specimens showed an absence of MLH1 and PMS2 proteins (Supplementary Fig. 2). Further, in Case 2, IHC of the initial BC showed an absence of MLH1 and PMS2 proteins, whereas no MMR proteins were absent in the second BC.

Cumulative risks of developing BC

The cumulative risks of one patient with LS developing BC were 4.35% at the age of 50 years, 8.70% at 60 years, and 21.5% at 70 years (Fig. 1).

Table 3 Clinicopathological characteristics of cases of breast cancer in females with Lynch syndrome

Case	Location	Stage	T	N	M	Pathologic type	Hormone receptor	HER2 receptor	MSI	Loss of MMR protein expression
1	Lt	2A	2	0	0	Med	ER–, PgR–	_	MSI-H	MLHL1, PMS2
2	Lt	2A	2	0	0	Sci	ER+, PgR+	–	MSI-H	MLHL, PMS2
2	Rt	1	1	0	0	Sci	ER+, PgR+	–	MSS	No absent
3	Lt	2B	2	1	0	Sci	ER+, PgR+	–	N.A.	N.A.
4	Rt	2A	2	0	0	Pap	ER+, PgR+	+	N.A.	N.A.
4	Lt	1	1	0	0	Sci	ER+, PgR+	–	N.A.	N.A.

Sci scirrhous carcinomas, *Med* medullary carcinoma, *Pap* papillotubular carcinoma, *Lt* left, *Rt* right, *N.A.* not available

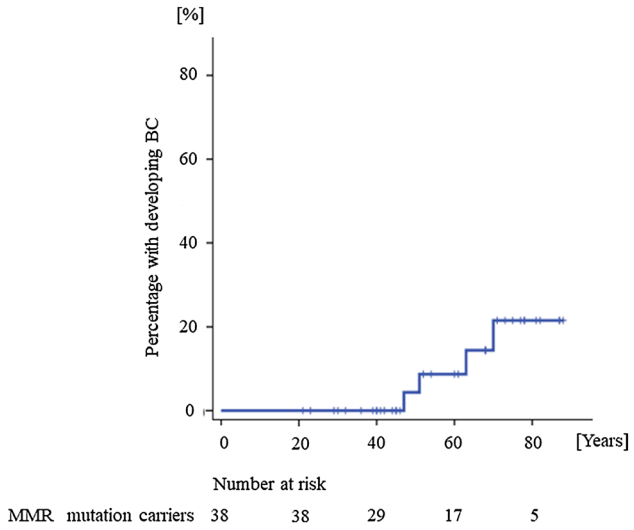

Fig. 1 The cumulative risk of developing breast cancer for females with Lynch syndrome

Discussion

Approximately 10% of all BCs are associated with inherited genetic mutations [7]. The majority of these are hereditary breast and ovarian cancers caused by mutations and common sequence variants in the *BRCA1* and *BRCA2* genes [8, 9]. BC due to other hereditary cancer syndromes, such as LS, Li–Fraumeni syndrome, or Cowden syndrome, is less common.

LS is the most common hereditary cause of colorectal and endometrial cancers. LS-associated cancers are attributable to defects in the DNA MMR genes such as *MLH1, MSH2, MSH6*, and *PMS2*. In the 25 LS families, there were 29 female carriers and 9 obligate mutation carriers. In the 38 females, 4 had BC, of which 2 had multiple contralateral BC. Two of the four females met a modified version of the Amsterdam II Criteria, whereas all met the revised Bethesda guidelines. Regarding diagnostic process for LS, most cases of LS were diagnosed from patients who developed common LS-associated cancer such as colon or endometrial cancer. Basically, screening criteria such as a modified version of the Amsterdam II Criteria and revised Bethesda guidelines are used for patients who develop colorectal cancers. Therefore, we are unsure about the number of patients who met these guidelines among patients with BC. There have been a few recent studies on BC associated with LS [10]. Win et al. [3] reported that BCs in LS were more likely to be poorly differentiated and to be ER, PgR, and HER2 negative. Our study showed that MMR-deficient BCs were poorly differentiated and HER2 negative (16.7%), but ER positive (83.3%) and PgR positive (83.3%).

Several studies are present on the frequency of MSI-H of BCs, with the reported incidence of MSI in BCs being 0–30% [11–16]. In LS, the frequency of MSI-H of BCs is reportedly not high enough although one of colorectal cancers is higher (Table 4) [17–22]. In the present study, two of the three BCs available for testing showed MSI-H. Although the mechanism of BC development in LS remains unknown, it is possible that an MMR defect accelerates the accumulation of mutations in BC-associated genes. Case 2 had metachronous bilateral BC. Interestingly, her initial BC showed MSI-H, whereas her second BC showed MSS. Further, IHC of the initial BC showed an absence of *MLH1* and *PMS2* proteins, whereas no MMR proteins were absent in the second BC. This suggested that the initial BC was caused by the MMR defect, whereas the second BC was sporadic cancer. Therefore, initial BC with MMR defect had rich TILs, whereas the second BC had poor TILs. Recently, immune checkpoint blockade, such as anti-programmed death-1 antibody, has improved clinical outcomes for patients with melanoma, lung cancer, or renal cell carcinoma [23]. However, the clinical benefit is limited to small population of patients with rich TILs [24]. Previously, MMR status predicted clinical benefit of immune checkpoint blockade with pembrolizumab for patients with colorectal cancer [25]. Immunotherapy may be a useful option for BC with MMR.

Two studies reported the mean age at the diagnosis of BC in LS to be 57.1 and 62.2 years [26, 27]; the finding of the present study (age, 63 years) was similar to the findings of these reports. This age is also similar to that of the general population in Japan. Thus, mutation carriers may be advised to participate in the general population BC screening programs, i.e., biannual examination and mammography from the age of 40 years [28].

Table 4 Summary of microsatellite instability of breast cancer in females with Lynch syndrome

Authors	Year	Age	MSI-H	ER	PgR	HER2
Müller et al. [18]	2002	N.D.	0% (0/27)	N.D.	N.D.	N.D.
de Leeuw et al. [19]	2003	43.9	63.6% (7/11)	N.D.	N.D.	N.D.
Blokhuis et al. [20]	2008	46	5.80% (4/69)	N.D.	N.D.	N.D.
Lotsari et al. [21]	2012	56	34.8% (8/23)	90.9% (20/22)	68.2% (15/22)	15% (3/20)
Grandval et al. [22]	2012	53.3	0% (0/14)	N.D.	N.D.	N.D.

N.D. no data

The cumulative risk of developing BC is reported to be higher in LS patients than in the general population [29, 30]. Previous studies demonstrated an increased risk of BC ranging from 2 to 18 folds compared with the general population [3]. Our results support the previous studies. To the best of our knowledge, there have been no previous reports on BC in LS patients from East Asia, and we believe the present study to be the first in East Asia. A limitation of our study was that the number of cases was very small. Although MSI testing or immunohistochemistry of MMR proteins is useful for screening to detect LS, genetic testing is necessary to confirm LS. Future studies are needed to further investigate the clinicopathological characteristics of and recommended surveillance for BCs in LS.

Conclusion

In our study, BC in Japanese females with LS was shown to be ER and PgR positive, HER2 negative, rich or moderate TILs, and MSI-H. For patients with LS, it is rare for the initial cancerous organ to be the breast. However, if patients with BC have a family history of cancers, BC specialists should suspect them to be possible carriers of LS.

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Compliance with ethical standards

Conflict of interest All authors have no conflict of interest.

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