Efficient Screening Strategy for Lynch Syndrome in Japanese Endometrial Cancer

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Lynch syndrome (LS) is an inherited disorder caused by a germline mutation in the DNA mismatch repair (MMR) genes and is associated with increased risk of various cancers, particularly colorectal cancer and endometrial cancer (EC). It is significant to identify LS in EC patients for prediction and prevention of the succeeding other associated cancers. However, useful LS screening guidelines for EC have not been established. The purpose of our study is to devise an efficient and practical screening strategy for LS in EC. We designed original criteria, named "APF criteria," with lenient terms (Age of onset < 50, or Personal or Family history of associated cancers) and applied it to unselected EC patients. We performed immunohistochemistry (IHC) and the methylation assay of MutL homolog 1 (MLH1) gene promoter using the tumors of patients who met our criteria, and thus selected "suspected LS" as the candidates for genetic analyses. Of 360 EC patients, 187 (51.9%) met the APF criteria, and the tumor specimens were available from 182 out of the 187 patients. IHC revealed that expression of at least one MMR protein was absent in cell nuclei of 54 (29.6%) tumors. Of 20 tumors lacking MLH1 protein expression, 14 cases were judged sporadic EC because of the hypermethylated MLH1 promoter. We thus selected 40 (11.1%) of 360 EC patients as "suspected LS." Our strategy that consists of clinical triage and the molecular analyses is expected to improve the screening efficiency and reduce the cost of LS identification in EC.

Introduction

Hereditary cancer syndrome, accounting for 5-10% of all cancers, are types of familial cancers that appear as a result of specific inherited genetic mutations and are often associated with multiple carcinogenesis or young age at onset. Lynch syndrome (LS) is caused by a germline mutation in the DNA mismatch repair (MMR) genes, including MutL protein homolog 1 (MLH1), MutS protein homolog 2 (MSH2), MutS protein homolog 6 (MSH6), and postmeiotic segregation increased 2 (PMS2) (Syngal et al. 2000; Hendriks et al. 2004; Senter et al. 2008), and thus the cancer risk of LS is inherited in an autosomal dominant pattern (Lindor et al. 2006; Win et al. 2013). When MMR function is impaired, the incidence of gene mutations increases, and the accumulation of carcinogenesis-related mutations leads to cancer development. Recent studies have indicated another etiology of LS; germline deletions in the epithelial cell adhesion molecule (EPCAM) gene lead to epigenetic inactivation of the MSH2 gene due to promoter hypermethylation (Tutlewska et al. 2013). The overview of LS remains unclear. LS is an inherited disorder that increases the risk of various cancer types, particularly colorectal cancer (CRC) and endometrial cancer (EC). LS-associated cancers include the cancer of stomach, ovaries, small intestine, liver, gallbladder ducts, upper urinary tract, brain, and skin. Recent studies have shown that women with LS account for 2-6% of all EC patients (Hampel et al. 2006; Ferguson et al. 2014) and that their lifetime risk of developing CRC is 43-48%, while that of developing EC is 40-62% (Aarnio et al. 1999; Lu and Broaddus 2005; Boilesen et al. 2008; Stoffel et al. 2009). In cases of women with LS who developed both primary CRC and gynecologic cancer, EC or ovarian cancer plays a role equal to or greater than that of CRC as a sentinel cancer of LS (Lu et al. 2005). In the 20 years following the diagnosis of EC, women with MMR gene mutations have a significantly increased risk of developing CRC or other associated cancers (Win et al. 2013). According to these studies, it is clinically significant to identify women with LS among EC patients to predict and prevent other associated cancers after EC treatment. It would also provide close blood relatives with an opportunity for the surveillance of LS-associated cancers.

Although oncologists often encounter women with LS

among EC patients, most are overlooked. An efficient screening strategy of LS is required, but the practical guidelines for identifying hereditary EC have yet to be established. The Amsterdam II criteria (Vasen et al. 1999) and the revised Bethesda guidelines (Umar et al. 2004) have been used as triage methods based on clinical data. The sensitivity of the Amsterdam II criteria is low due to its highly strict requirements, and the evaluation of its specificity is divided (Syngal et al. 2000; Lipton et al. 2004; Vasen et al. 2007). The revised Bethesda guidelines have a sensitivity of 82-94% in patients with CRC (Syngal et al. 2000; Piñol et al. 2005), but their utility in other associated cancers is unknown. The Society of Gynecologic Oncologists (SGO) Criteria (Lancaster et al. 2007), which followed the revised Bethesda guidelines, were released in 2007 as a triage method for gynecologic cancers. The effectiveness of the SGO criteria is now being inspected.

Microsatellite instability (MSI) and immunohistochemistry (IHC) testing are used for molecular analyses of LS, and their sensitivity and specificity have been reported (Lu et al. 2007; Modica et al. 2007; Resnick et al. 2009; Moline et al. 2013). When there are abnormalities in the MMR pathway, replication errors in the microsatellite, a repeated sequence present in the DNA, become difficult to repair, thereby resulting in MSI. IHC is a method for assessing the expression of MMR proteins and predicts the MMR gene disorder corresponding to MMR protein expression loss. Addition of the methylation assay to the molecular analyses is effective for narrowing down suspected LS patients (Hampel et al. 2006; Gausachs et al. 2012; Leenen et al. 2012).

Some researchers suggest that universal screening (US), which applies molecular analyses to all patients with EC, should be implemented as a highly sensitive screening method (Hampel et al. 2006; Moline et al. 2013). However, because the perspectives of LS are ambiguous and guidelines for surveillance have not been established, it is difficult to determine the clinical usefulness of US at this time. On the other hand, screening strategies that consist of clinical data and molecular analyses are needed (Garg and Soslow 2009; Kwon et al. 2011), and these strategies may lead to optimization and cost reductions for identifying LS. The incidences of LS-associated cancers differ among races, ethnicities, and geographical regions (Benatti et al. 1993; Park et al. 1999), but the research on the clinical distribution and identification strategies of LS in unselected EC patients have been reported only in Western countries. Understanding the regional characteristics and clinical features of LS in EC patients would lead to more appropriate identification strategies and surveillance methods, but no investigative research covering the entire population of EC patients has been conducted in East Asia.

The purpose of our study is to elucidate the clinical distribution and characteristics of LS in Japanese EC patients and to devise an efficient screening strategy that can be widely used in daily clinical practice.

Methods

Study population and procedures

Total 360 EC patients who were diagnosed and treated at Akita University Hospital between January 2003 and December 2013 were identified retrospectively. All study participants provided written informed consent in the prescribed document approved by an ethics review board. All of the patients were Asians living in Japan. The patients' clinical data such as age, personal medical history, and family history were collected from medical records. We designed original criteria, named "APF criteria," using simple and lenient terms (Age of onset < 50, or Personal or Family medical history of associated cancers), and applied it to unselected EC patients (Table 1). The patients satisfying one or more of the three criteria are considered to have met the criteria. The patients who did not meet the APF criteria were considered as "probable sporadic EC." Performing molecular analyses, IHC and an optional MLH1 methylation assay, on the tumors of patients who met our criteria, we selected "suspected LS" as the candidates for genetic analyses.

Immunohistochemistry

IHC was performed as primary molecular analysis to assess MMR protein expression (MLH1, MSH2, MSH6, PMS2), according to standard procedure (Matthews et al. 2008; Backes et al. 2009; van Lier et al. 2010). An appropriate paraffin-embedded tissue was cut at 4 μm. The tissue sections were deparaffinization in xylenes and rehydrated in graded alcohols. Subsequently, antigen retrieval was performed in 10 mmol/L Tris-EDTA buffer (pH 9.0) in microwave oven for 20 minutes. These sections were allowed to cool at room temperature. Then, the primary anti-bodies were applied overnight at 4°C. The primary antibodies were MLH1 (clone ES05; dilution 1:50; Dako), MSH2 (clone FE11; dilution 1:50; Dako), MSH6 (clone EP49; dilution 1:50; Dako) and PMS2 (clone EP51; dilution 1:40; Dako). Antigen-antibody reaction was visualized with the Envision kit (Dako). The slides were counterstained with hematoxylin. Adjacent normal endometrium and lymphocytes in the slides were used as

Table 1. APF criteria.
(Age, Personal medical history, Family medical history)

• EC patient diagnosed less than 50 years of age
• EC patient with a synchronous or metachronous Lynch syndrome associated tumors*, regardless of age
• EC patient with at least one first or second degree relative** with Lynch syndrome associated tumors*, regardless of age

*Lynch syndrome associated tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain (usually glioblastoma as seen in Turcot syndrome) tumors, sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome, and carcinoma of the small bowel.
**First and second degree relatives are parents, siblings, aunts, uncles, nieces, nephews, grandparents and grandchildren.

internal positive control. The stained slides were evaluated by two or more gynecologic oncologists. We judged the complete absence of nuclear staining in the tumor cells as MMR protein expression loss.

MLH1 promoter hypermethylation assay

MLH1 promoter hypermethylation assay was performed on the tumors lacking the expression of MLH1 alone or both MLH1 and PMS2. The tumor DNA was extracted from mapped formalin-fixed, paraffin-embedded tissue sections to provide tumor samples for assay. The SALSA MS-MLPA (Methylation-Specific Multiplex Ligation-Dependent Probe Amplification) kit ME011 MMR genes (MRC-Holland, Amsterdam, The Netherlands) was used for study to detect aberrant CpG islands methylation in the promoter of MMR genes, including 5 probes for MLH1. MS-MLPA assay were performed as described by the manufacturer. The dichotomization threshold to distinguish methylated versus unmethylated samples was established at 20% based on a previous study (Gausachs et al. 2012). Tumors lacking MLH1 protein expression with MLH1 promoter hypermethylation were judged sporadic EC (Pérez-Carbonell et al. 2010).

Prediction of corresponding MMR gene mutations

The MMR proteins function as heterodimers; MSH2 forms a heterodimer with MSH6 or MSH3, and MLH1 forms a heterodimer with PMS2 or PMS1 (van Lier et al. 2010; Moline et al. 2013). Thus, the corresponding MMR gene mutations were predicted from loss of MMR protein expression (see Table 2). Tumors retaining MMR protein expression were judged sporadic EC.

Selection of suspected LS patients

Patients with predicted mutations of MSH2, MSH6, or PMS2 were selected as suspected LS. Patients with tumors lacking MLH1 expression and MLH1 promoter hypermethylation were selected as suspected LS.

Evaluation of clinical criteria

We evaluated the rate of suspected LS patients who satisfied the Amsterdam II and SGO criteria.

Statistical analysis

The characteristics of suspected LS were statistically compared with those of probable sporadic EC using the Chi-square test or two-sample t-tests. Statistical significance was defined at values of $P < 0.05$.

Results

Of the 360 EC patients, 187 (51.9%) met the APF criteria (Fig. 1). Of these 187 patients, 58 were diagnosed with EC at < 50 years of age, 33 had both EC and LS-associated cancers, and 146 had at least one of first- or second-degree relatives with LC-associated cancers. IHC was performed on the tumor samples of the 182 patients for whom tissue specimens were available, and we were able to evaluate all of these samples.

In 54 (29.6%) of 182 cases, the expression of at least one MMR protein was completely absent in the nuclei of tumor cells. The MLH1 promoter hypermethylation assay was conducted in 20 tumors lacking the expression of MLH1 alone or both MLH1 and PMS2. This assay revealed that the MLH1 gene was hyper-methylated in 14 (70%) of 20 tumors, and thus these 14 cases were considered as sporadic EC. Using our screening strategy, we selected 40 (11.1%) of 360 EC patients as suspected LS.

On the basis of immunohistochemical MMR expression patterns (Table 2), the corresponding MMR gene mutations were predicted: six cases of MLH1, 13 cases of MSH2, five cases of MSH6, 10 cases of PMS2, one cases of MLH1 or MSH2, and five cases of MLH1 or MSH6.

Of all 360 EC patients, seven (1.9%) fulfilled the Amsterdam II criteria, 100 (27.8%) satisfied the SGO 5-10% criteria, and both of these groups certainly met the APF criteria (Table 3). Of the 40 genetic testing candidates (suspected LS with EC) selected by our screening strategy, four (10%) fulfilled the Amsterdam II criteria and 25 (65%) satisfied the SGO 5-10% criteria.

The clinical and pathological characteristics of the "probable sporadic EC" group and the "suspected LS" group are shown in Table 4. Of 40 suspected LS patients, six (15%) had a personal medical history of stomach cancer, and 18 (45%) had a family medical history of stomach cancer. In suspected LS group, the stomach cancer incidence among the patients and their family was similar to that of CRC, and significantly higher than that in probable sporadic EC group.

Table 2. Predicted MMR gene mutations associated with protein expression patterns.

		Predicted MMR gene mutation					
		MLH1	MSH2	MSH6	PMS2	MLH1 or MSH2	MLHI or MSH6
MMR Protein expression	MLH1	−	+	+	+	−	−
	MSH2	+	−	+	+	−	+
	MSH6	+	+ or −	−	+	+ or −	−
	PMS2	+ or −	+	+	−	+ or −	+ or −

+ : presence of nuclear staining of tumor cells (as well as in normal cells).

− : absence of nuclear staining of tumor cells (and presence of nuclear staining in adjacent normal cells served as internal positive controls).
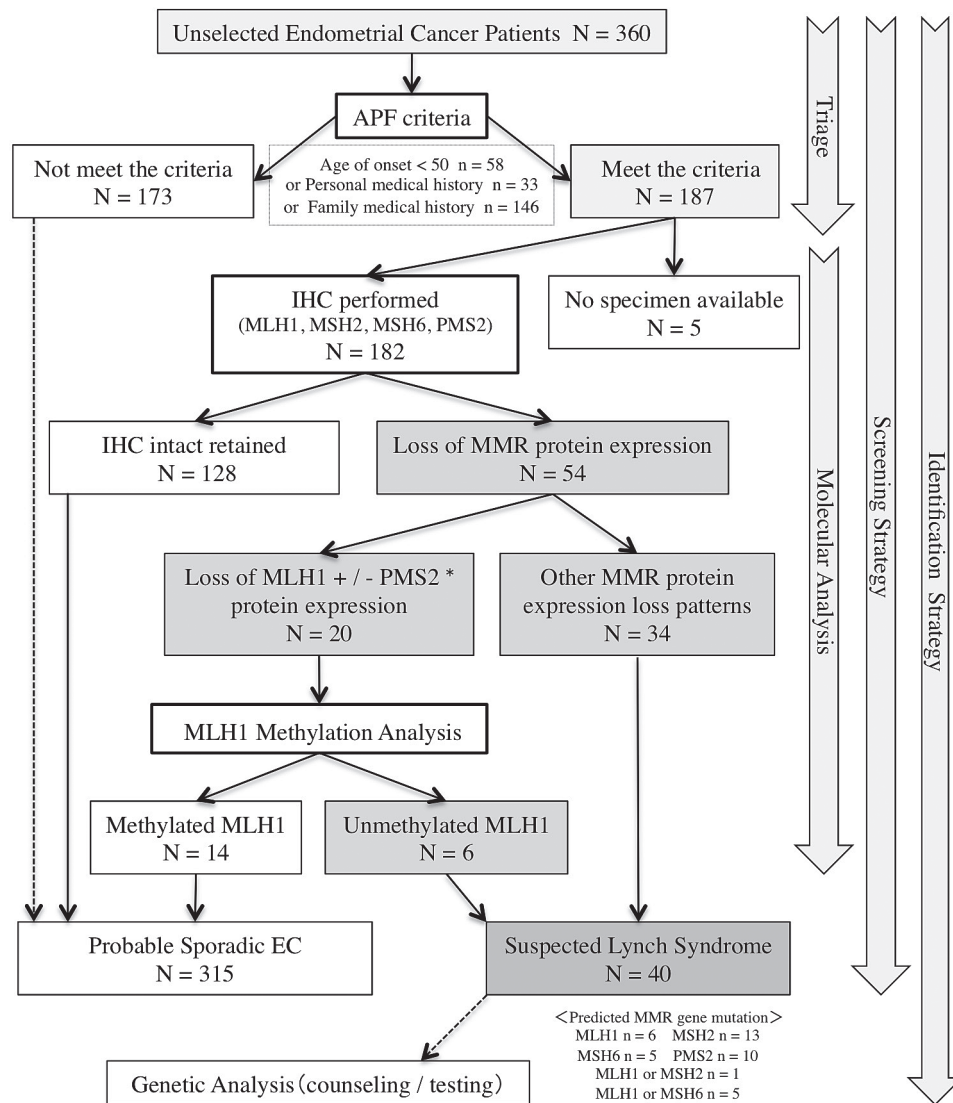
Fig. 1. Summary of Lynch syndrome screening strategy.
EC, endometrial cancer; IHC, immunohistochemistry.
*[MLH1 +/− PMS2] means MLH1 alone or both MLH1 and PMS2.

Table 3. Evaluation of clinical criteria.

Clinical Criteria for LS	Total EC N = 360	Suspected LS N = 40
Meet the Amsterdam II criteria	7 (1.9%)	4 (10%)
Meet the SGO 5-10% criteria	100 (27.8%)	26 (65%)
Meet the APF criteria (original)	187 (51.9%)	40 (100%)

EC, endometrial cancer; LS, Lynch syndrome; SGO, Society of Gynecologic Oncologists; APF, original criteria (see Table 1).

Discussion

Clinicians have been overlooking most of LS cases accounting for 2-6% of EC patients (Hampel et al. 2006; Ferguson et al. 2014). To efficiently identify LS in all EC patients and to provide them with appropriate surveillance, we seek a sensible screening strategy for clinical practice.

The incidence and distribution of LS-associated cancers differ among races and countries (Benatti et al. 1993; Park et al. 1999). Some reports have indicated that gastric cancer in LS patients occurs more frequently in East Asia than in Western countries (Lindor et al. 2006; Vasen et al. 2013). The clinical characteristics of LS in Japanese EC patients have been reported remarkably (Hirai et al. 2008),

Table 4. Characteristics of "probable sporadic endometrial cancer" and "suspected Lynch syndrome".

	Probable Sporadic EC N = 315	Suspected LS N = 40	P
Median age at diagnosis of EC	60.0 (28-89)	55.5 (35-77)	
< 50 years at diagnosis of EC	41 (13.0%)	13 (32.5%)	0.49[†]
Mean BMI	24.5	24.3	0.72[†]
Personal history of LS-associated cancers*	18 (5.7%)	14 (35.0%)	< 0.01[†]
CRC	7 (2.2%)	8 (20.0%)	< 0.01[†]
stomach cancer	8 (2.5%)	6 (15.0%)	< 0.01[†]
Family history of LS-associated cancers**	111 (35.2%)	33 (82.5%)	< 0.01[†]
EC	11 (3.5%)	2 (5.0%)	0.65[†]
CRC	25 (7.9%)	16 (40.0%)	< 0.01[†]
stomach cancer	68 (21.6%)	18 (45.0%)	< 0.01[†]
Histology			
Endometrioid	261 (82.9%)	34 (85.0%)	0.91[‡]
Non-endometrioid	54 (17.1%)	6 (15.0%)	
Tumor grade			
1 and 2	233 (74.0%)	31 (77.5%)	0.77[‡]
3	82 (26.0%)	9 (22.5%)	
FIGO Stage			
I and II	237 (75.2%)	34 (85.0%)	0.24[‡]
III and IV	78 (24.8%)	6 (15.0%)	

EC, endometrial cancer; CRC, colorectal cancer; LS, Lynch syndrome; FIGO, International Federation of Gynecology and Obstetrics.
*Patient with a synchronous or metachronous Lynch syndrome associated cancers.
**Patient has a first- or second-degree relative with Lynch syndrome associated cancers.
[†]Comparison of "probable sporadic EC" and "suspected LS" using 2 sample significant test.
[‡]Comparison of "probable sporadic EC" and "suspected LS" using significance test for 2 × 2 tables.

but their study had some clinical bias in the selection of candidates for genetic analysis. In Asia, no studies on the clinical distribution and characteristics of LS among all EC patients have been reported. In our study, many patients had a personal or family medical history of stomach cancer. This characteristic was particularly remarkable in the suspected LS group. The difference of stomach cancer incidence in the family between suspected LS and probable sporadic EC group was statistically significant, but the correlation between stomach cancer and LS has not been verified by molecular or genetic analysis (Table 4). When identifying LS in Japanese population, we should consider the high incidence of stomach cancer. Furthermore, when using clinical triage we should consider the bias it brings.

On the hereditary cancer triage, age of onset, personal medical history, and family medical history are important factors. However, there are difficulties and limitations in obtaining a thorough family history in daily clinical practice. Before the corresponding genes of LS became widely acknowledged, the Amsterdam II criteria were used for the clinical diagnosis. In our study, only seven (1.9%) of all 360 EC patients satisfied the Amsterdam II criteria. On the basis of our results, the sensitivity of the Amsterdam II criteria must be low. Of these seven patients, only four (57%) were lacking MMR protein expression and the specificity

of the criteria was not high. The SGO criteria released in 2007 are being now inspected. The sensitivity of the SGO 5-10% criteria for MMR gene mutation carrier is reportedly to be 85.7-93% (Ryan et al. 2012; Buchanan et al. 2014). On the other hand, Bruegl et al. (2014a) skeptically reported that the sensitivity of the SGO 5-10% criteria for candidates for genetic testing (probable LS with EC) is 32.6%. The sensitivity of the criteria is influenced by positive selection (MMR gene mutation carrier or candidates for genetic testing) and the population selection. Individuals with the MSH6 or PMS2 germline mutation present with EC at a relatively older age and have relatively weak family histories of LS-associated cancers (Hendriks et al. 2004; Senter et al. 2008). Thus, they are more likely to be missed even by the SGO 5-10% criteria than those with the MLH1 or MSH2 mutation. For higher triage sensitivity, we set the APF criteria with lenient terms and applied it to all 360 EC patients as the first triage of LS.

MSI and IHC testing are the most commonly used molecular analyses for tumors of LS patients. At least 90% of EC patients who carry the MMR gene mutation exhibit MSI (Hampel et al. 2006). Approximately 20% of sporadic EC patients also exhibit MSI, and many cases of them are caused by hypermethylation of the MLH1 promoter region (Esteller et al. 1998). In CRC, it has been reported that

MSI is a predictive factor for response to antineoplastic agents and is associated with their prognosis (Black et al. 2006; Vasen et al. 2007), but these issues have not been elucidated in EC patients. IHC exhibits a high sensitivity of approximately 95% (Hampel et al. 2006; Resnick et al. 2009) and is commonly used in clinical practice, but its sensitivity and specificity are influenced by the assessment quality of technician (Modica et al. 2007). When loss of MMR protein expression was suspected, multiple gynecologic oncologists performed assessments to ensure quality. Studies have indicated that MSI and IHC testing have almost the same sensitivity when used as LS screening analyses (Lu et al. 2007; Modica et al. 2007). We chose to use IHC in our study for the following three reasons: 1) IHC is relatively inexpensive and can be conducted at many clinical facilities, whereas MSI testing can be performed at limited facilities and is relatively expensive; 2) IHC leads to the prediction of a corresponding germline mutation, which makes it possible to select suitable genetic testing; and 3) IHC is more highly capable than MSI for detecting MSH6 germline mutation (Hampel et al. 2006; Resnick et al. 2009). The MSH6 mutation is found more frequently in EC patients than in CRC patients (Hendriks et al. 2004). When IHC is performed on the tumors taken from all EC patients, 75-90% of tumors with loss of MLH1 protein expression are sporadic EC with MLH1 gene promoter hypermethylation (Bruegl et al. 2014b; Buchanan et al. 2014). We performed methylation assays on the 20 tumors with loss of MLH1 alone or MLH1 and PMS2 protein expression. We subsequently identified MLH1 promoter hypermethylation in 14 (70%) of them. In some studies, the methylation assay was replaced by a clinical refinement method based on MLH1 germline mutation carrier characteristics such as young age at onset and extensive family history (Backes et al. 2009, 2011), but the validity of this tactic has yet to be verified.

Of the patients recommended to undergo genetic counseling, only 20-50% actually do so (Backes et al. 2009; Batte et al. 2014). The reasons not to accept genetic analyses (genetic counseling/genetic testing) primarily include medical cost, anxiety over the results, lack of risk awareness, and indifference to hereditary cancers (Backes et al. 2011; Batte et al. 2014). To promote genetic analyses and proactive medical interventions, the following is required: subsidization of the medical cost, privacy protection, consolidation of medical knowledge, and the availability of an established information system. MMR germline mutations occur as insertions and deletions at the base level or as duplications and deletions at the exon level. These can be detected by genetic testing such as direct sequencing and MLPA. Direct sequencing is suitable for detecting aberrations at the base level, and MLPA is highly efficient at detecting aberrations at the exon level. Therefore, genetic testing methods should be selected according to subjects. Suspected LS patients without germline mutations were investigated in a large-scale study of CRC. Reports have

shown that the risk of LS-associated cancers is higher in families of these patients than in the general population (Pérez-Carbonell et al. 2012; Rodríguez-Soler et al. 2013). These reports suggest that there may be indeterminate LS-related genetic mutations that cannot be identified by standard genetic testing. Even if germline mutation in the MMR genes is not detected, the possibility of hereditary cancer syndromes including LS cannot be completely ruled out.

Many guidelines and studies recommend US in which molecular analyses are implemented in all CRC patients (EGAPP Working Group 2009; Vasen et al. 2013). Some researchers recently recommended the use of US in EC patients as well (Hampel et al. 2006; Moline et al. 2013). However, the implementation of US in EC patients has the following three problems. The first problem is its cost-effectiveness. Considering the fact that LS accounts for 2-6% of all EC patients (Hampel et al. 2006; Ferguson et al. 2014), opinions on the cost-effectiveness of US are divided. In a comparative study using a simulation model of several LS screening strategies, the strategy that adds an IHC assessment for EC patients who have at least one first-degree relative with LS-associated cancer has better cost-effectiveness than US (Kwon et al. 2011). However, this strategy is at the sacrifice of sensitivity. The second problem is that patients selected by US have particularly low ratio to undergo genetic analyses. EC patients who have little or no personal or family history of LS-associated cancers tend not to undergo expensive analyses (Backes et al. 2009; Batte et al. 2014). If the social environment and medical systems that provide genetic information and support for patients are not properly maintained, the benefits that US can bring to patients will be much less extensive than the theoretical benefits. The third problem is that the positive predictive value of US is lower than those of other screening strategies (Buchanan et al. 2014; Ferguson et al. 2014), implying that the ratio of patients with suspected LS without germline mutations is relatively high. In the group of suspected LS patients without germline mutations, the incidence of LS-associated cancers is lower than that in the group of LS patients, but significantly higher than that in the general population. This tendency suggests that unknown hereditary cancer syndromes and sporadic cancer are intermixed in this group. Some reports about CRC suggest that patients in this group should be considered as suspected LS and undergo the surveillance for LS patients (Pérez-Carbonell et al. 2012; Rodríguez-Soler et al. 2013). Management guidelines for EC patients in this group should be provided.

The APF criteria, an original triage tactic of LS, have higher sensitivity than those of the SGO 5-10% criteria. We believe that the strategy consisting of the APF criteria and molecular analyses leads to more efficient LS screening in EC patients than US and that it enables the implementation of appropriate management. Many studies have indicated that US consisting of IHC and additional methylation

assays could select about 10% of all EC patients as suspected LS (Batte et al. 2014; Bruegl et al. 2014a; Buchanan et al. 2014). Using our strategy, we selected 40 (11.1%) of the 360 EC patients as candidates for genetic testing (suspected LS). The screening ratio of our strategy is almost equal to that of US in the selection of suspected LS patients. The analysis cost of our screening strategy is much less than that of US, and our strategy may have a higher positive predictive value than US. The effective use of clinical data may lead to persuasive information and increase the implementation rate of genetic analysis, resulting in a larger number of patients and family members undergoing surveillance. However, further studies are required to evaluate the validity of triage using clinical information prior to the molecular analyses. Whether medical intervention is necessary for EC patients who have no suspected clinical factors of LS should be investigated.

The maximum benefit of identifying LS in EC patients is to obtain surveillance and preventive medicine opportunities of LS-associated cancers. Clinicians are able to evaluate the possibility of LS by understanding a brief overview and regional characteristics of LS and by accurately assessing the patients' personal and family histories. The clinicians will provide individualized service and advice to suspected LS patients by providing appropriate information about the benefits and limitations of genetic analyses and surveillance. We believe that mutational analyses of the MMR gene should be performed in cases of suspected LS. However, when we perform genetic testing in the absence of basic research and surveillance methods, ethical problems would occur in patients and their families. This study has the significance of being the essential foundation for genetic diagnosis. Further studies including prospective research are required to establish a strategy for identifying LS in EC patients.

In conclusion, our study showed a portion of the clinical characteristics of suspected LS in Japanese EC patients. Our screening strategy, which consists of highly sensitive clinical triage and specific molecular analyses, is expected to improve the screening efficiency and reduce the identification cost of LS.

Conflict of Interest

The authors declare no conflict of interest.

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