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Clinical impact of genetic alterations of *CTNNB1* in patients with grade 3 endometrial endometrioid carcinoma

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Abstract

To identify prognostic factors in patients with grade 3 (high-grade) endometrial endometrioid carcinoma, we evaluated the spectrum of genomic alterations and examined whether previously reported molecular subtypes of endometrial carcinoma were adapted to clinical outcome prediction. Seventy-five Japanese patients with grade 3 endometrial endometrioid carcinoma, who underwent a potentially curative resection procedure between 1997 and 2018 at the National Cancer Center Hospital, were included. We classified the patients into four risk groups of the disease based on the Proactive Molecular Risk Classifier for Endometrial Cancer. Genomic alterations in *PTEN*, *ARID1A*, *TP53*, and *PIK3CA* were detected in more than 30% of the patients. Overall survival and recurrence-free survival of patients with genomic alterations in *CTNNB1* were poorer than those of patients with wild-type *CTNNB1* ($p = 0.006$ and $p = 0.004$, respectively). Compared with that of alterations prevalent in Caucasians, the frequency of genomic alterations in *POLE* and *TP53* was higher in our study than in The Cancer Genome Atlas dataset ($p = 0.01$ and $p = 0.01$, respectively). The tendency for recurrence-free survival in the *POLE* exonuclease domain mutation group was better than that in the *TP53* mutation and mismatch repair-deficient groups ($p = 0.08$ and $p = 0.07$, respectively), consistent with the Proactive Molecular Risk Classifier for Endometrial Cancer risk classifier definition. The *CTNNB1* mutation is a potential

novel biomarker for the prognosis of patients with grade 3 endometrial endometrioid carcinoma, and prognosis classification using Proactive Molecular Risk Classifier for Endometrial Cancer may help screen Japanese patients with the disease.

KEYWORDS
CTNNB1, genetic alterations, grade 3 endometrial endometrioid carcinoma, prognosis, ProMisE

1 | INTRODUCTION

The global prevalence of EC is on the rise, with over 380,000 new cases being diagnosed in 2018.[1] EC, the most commonly observed gynecologic malignancy regardless of ethnicity,[1,2] is categorized as estrogen-dependent type I (80%–90%), including grade 1 and grade 2 (low-grade) endometrial endometrioid carcinoma (G1EEC and G2EEC, respectively), and estrogen-independent type II (10%–20%). Type II EC has a significantly worse outcome than type I EC.[3] In addition, type II ECs are conventionally divided into two groups: (1) grade 3 (high-grade) endometrial endometrioid carcinoma (G3EEC), and (2) nonendometrioid carcinoma (serous carcinoma, clear cell carcinoma, and uterine carcinosarcoma).[4,5] The prognosis and molecular features of the two groups differ.[6,7] However, similar survival outcomes of the two groups have been reported by some case series,[6] although G3EEC was reportedly associated with a better OS than nonendometrioid carcinoma in a retrospective review of data obtained from the Surveillance, Epidemiology and End Results Program.[8] Therefore, the prognosis of, and optimal treatment for, G3EEC remain controversial. Although several studies have focused on G3EEC in Western countries,[9,10] only one study has focused on G3EEC in Asia.[11] Therefore, there is a need to identify the significant prognostic factors associated with clinical outcomes in Asian patients with G3EEC and elucidate different clinicopathological factors and somatic mutational patterns according to ethnicity.

Recent advances in cancer genome research have led to the elucidation of genomic alteration profiles of G3EEC in Caucasians via TCGA.[7] Data from TCGA have indicated that the frequencies of mutations of *PTEN* and *TP53* in G3EEC are higher than those in grades 1 and 2 EEC.[7] In addition, the frequencies of *PTEN*, *ARID1A*, and *PIK3CA* mutations in G3EEC are higher than those in serous carcinoma.[6,12] Furthermore, the distribution of somatic mutations in cancer-related genes in Asian patients with G3EEC remains unknown and, because of the rare histological types, validation studies confirming that somatic mutations are associated with clinical outcomes are scant.[13,14] Therefore, the frequency of oncogenic mutations in Japanese patients with G3EEC, as one of the Asian patient subpopulations, needs further clarification, and the association between genomic alteration classification and clinical outcomes requires further verification.

The Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) has shown potential as a standard for the risk stratification of patients with EC.[15] In ProMisE, four risk groups are indicated as follows: (1) with *POLE* mutations, (2) mismatch repair (MMR)-deficient (without MMR immunohistochemical [IHC] staining, or microsatellite instability-high), (3) with *TP53* mutations (identified via targeted or whole-exome sequencing using clinical specimens, or abnormalities in p53 staining via IHC staining), and (4) NSMP.[9] Although the prognostic impact of ProMisE has been validated in several reports,[16,17] it is unclear whether these analyses can be applied to Asian patients with G3EEC.

In this study, we aimed to investigate the prognostic factors in patients with G3EEC and to compare the genomic alteration profiles of Japanese patients with G3EEC with those of Caucasians. Additionally, we evaluated the utility of previously reported molecular subtypes of endometrial carcinoma (ProMisE classification), which were originally developed for Caucasian patients with G3EEC.

2 | MATERIALS AND METHODS

2.1 | Clinical profiles of 74 patients with G3EEC

Ninety-four patients with G3EEC who underwent surgery after diagnosis between 1997 and 2018 at our hospital were enrolled. The Institutional Review Board of the National Cancer Center Research Institute approved this study (2017-331). For patients diagnosed between 2000 and 2018, the general requirements for informed consent for the use of their samples in research was obtained at their first visit to our hospital. Information obtained in our study using samples collected after obtaining general informed consent from participants has been summarized in the website of our hospital. Patients were free to revoke their presumed consent at any time point. We used samples from only patients who did not revoke their consent. Similarly, we informed patients treated before 2000 that the information summary of our study is published on the official website of our hospital. Patients who refused to provide consent for the use of their residual samples were excluded from this study. Of the 94 patients, 74 patients whose sequencing data met quality control criteria were selected for the study (Figure S1). Clinical data, including age, cancer stage (as defined by the International Federation of Gynecology and Obstetrics in 2008[18]), pathological factors, and survival time after primary surgery, were retrospectively obtained for each patient. The period from the date of surgery to the last known visit or confirmation of recurrence or death was defined as the follow-up time and time to an event, respectively. Recurrence-free survival was calculated from the date of primary surgery to the date of first recurrence or death from any cause. OS was calculated from the date of primary surgery to the date of death from any cause. At least two gynecological pathologists confirmed the pathological

diagnoses. During the pathology review, the pathologists evaluated all sections of the tumors and carefully excluded the possibility of other types of high-grade ECs and carcinosarcomas. These distinctions were based on the morphological and immunohistochemical findings: the presence of slit-like spaces, a high variability in nuclear pleomorphism, and p16 block positivity favored the diagnosis of serous carcinoma. In addition, lower-grade glandular areas, variant differentiation (squamous or mucinous), normal p53 expression, and abnormal MMR expression favored the diagnosis of endometrioid carcinoma. High-grade carcinomas with ambiguous features were not included in this study.

2.2 | Deep sequencing for hotspot regions in 50 cancer-related genes, including all exons of *PTEN*, *ARID1A*, *TP53*, *POLE*, *PIK3R1*, and *PPP2R1A*, and the *TERT* promoter

Purified genomic DNA obtained from formalin-fixed, paraffin-embedded tumor tissues (50 ng) was used for library construction, using the Ion AmpliSeq Cancer Hotspot Panel v2 (Thermo Fisher Scientific), as previously reported.[19] This panel targets ~2800 COSMIC mutational hotspot regions of 50 cancer-related genes, including *PIK3CA*, *KRAS*, *ATM*, *FBXW7*, *CTNNB1*, *KIT*, and *SMAD4*. An Ion AmpliSeq Custom Panel, designed for all exons of *PTEN* (coverage rate: 89.2%), *ARID1A* (coverage rate: 97.3%), *TP53* (coverage rate: 100%), *POLE* (coverage rate: 100%), *PIK3R1* (coverage rate: 99.6%), *PPP2R1A* (coverage rate: 100%), and *TERT* (promoter region) using the Ion AmpliSeq™ Designer (https://www.ampliseq.com), was also used (Solution ID: IAD191594_167). Sequencing was performed using the Ion Proton platform (Thermo Fisher Scientific). For quality control, samples with a mean read depth of coverage more than 1000 and base quality score of 20 (≤1% probability of being incorrect), which accounted for 80% of the total reads, were selected.

2.3 | Detection of copy number alterations using the TaqMan assay

To identify copy number alternations, real-time PCR was performed using the TaqMan copy number assays, including those for *MYC* (ID Hs01764918_cn), *ERBB2* (ID Hs02803918_cn), *CCNE1* (ID Hs07158517_cn), and *PIK3CA* (ID Hs02202946_cn), on the ABI 7900HT real-time PCR system (Applied Biosystems). We selected four genes, namely *MYC*, *ERBB2*, *CCNE1*, and *PIK3CA*, because they had been associated with a poor prognoses in patients with EC[7,20] and have a copy number alteration frequency greater than 5% in the TCGA dataset of patients with G3EEC.[7] Genome data were analyzed for copy number alternations using the ABI PRISM 7900HT Sequence Detection Software CopyCaller v2.1 (Thermo Fisher Scientific). Amplification was defined using the copy number assay as the presence of more than eight copies of *PIK3CA* and more than four copies of *MYC*, *ERBB2*, or *CCNE1*.

2.4 | IHC analysis and interpretation

All IHC tests, including those for p53 (DO7, prediluted, Dako, Glostrup, Denmark), ARID1A (rabbit polyclonal, 1:2000, Sigma), PMS2 (EP51, prediluted, Dako), and MSH6 (EP49, prediluted, Dako), were performed as described in our previous study.[21,22] Furthermore, an IHC test for CTNNB1, the primary antibody of β-catenin (clone14, 1:500; BD Bioscience), was performed in this study. The term "aberrant p53 staining pattern" refers to either a strong and diffuse nuclear staining pattern (>80% of carcinoma cells) or completely negative ("null pattern") staining pattern of carcinoma cells, with staining of the adjacent nontumor cells serving as an internal positive control. The staining pattern of wild-type tumor cells was weak and heterogeneous. The loss of ARID1A nuclear expression in tumor cells was classified as either homogeneous (negativity in almost all tumor cells) or heterogeneous (regional negativity). Because IHC for PMS2 and MSH6 can reportedly be used instead of the four antibody panels (MLH1, MSH2, MSH6, and PMS2) for MMR-deficient screening,[23] for the purpose of this study, the MMR-deficient status was defined as the complete loss of nuclear staining for PMS2 and/or MSH6 proteins. Internal positive controls with intact nuclear staining included the adjacent normal mucosa, stromal cells, and inflammatory cells.

2.5 | Classification of oncogenic/ actionable mutations

Data analysis was carried out using Torrent Suite Software v5.0.4 (Thermo Fisher Scientific). First, somatic mutations were selected using the following criteria[19]: (1) variant allele frequency of somatic mutations in tumor tissues >4%, (2) removal of single-nucleotide polymorphisms with a threshold allele frequency value ≥0.01 in either the NHLBI GO Exome Sequencing Project (ESP6500; http://evs.gs.washington.edu/EVS/) or the Integrative Japanese Genome Variation Database (iJGVD, 20181105) (https://ijgvd.megabank.tohoku.ac.jp/)[24], and (3) registration of mutations as "pathogenic/likely pathogenic variants" in ClinVar[25] or "oncogenic/likely oncogenic variants" in OncoKB (http://oncokb.org) databases[26] using OncoKB-annotator (accessed on 14 April 2020). In the current study, gene aberrations with evidence levels 1A-3B registered in OncoKB were identified as candidate actionable mutations for molecular-targeted drugs.[27] Finally, all selected variants were manually inspected using the Integrative Genomics Viewer (http://www.broadinstitute.org/igv/).[28]

2.6 | Classification of four ProMisE molecular groups in our cohorts

ProMisE divided patients into four groups: (1) *POLE* mutations, (2) MMR deficient, (3) *TP53* mutations, and (4) NSMP. The steps for molecular classification, which were based on the WHO classification

code,[29] were as follows. First, tumors were assessed for _POLE_ EDMs. In our study, _POLE_ EDMs were defined as the five most common _POLE_ mutations: P286R (exon9), V411L (exon13), S297F (exon9), A456P (exon14), and S459F (exon14).[30] A large majority of mutations outside these EDMs were characterized as non-_POLE_ EDMs. Only _POLE_ EDMs were categorized into a _POLE_ mutation group. Next, the presence of MMR proteins was assessed using PMS2 and MSH6 via an IHC test.[16] Finally, tumors were assessed for p53 null/missense mutations, yielding four subgroups: _POLE_ EDM, MMR-D, _TP53_ mutants, and _TP53_ wild-type.[31]

2.7 | Somatic mutation profiles of 132 patients with G3EEC in TCGA database

We selected 132 cases of G3EEC registered in TCGA. Somatic mutations called from whole genome sequencing and whole-exome sequencing data available in TCGA were retrieved in the mutation annotation format via the cBioPortal for Cancer Genomics (http://www.cbioportal.org).

2.8 | Statistical analysis

R 3.3.1 (R Foundation) and JMP version 8.0.1 (SAS Institute) were used for statistical analyses. Fisher's exact test was used to investigate the association between genomic alterations and clinicopathological factors using R software. Cumulative survival was calculated using the Kaplan–Meier method, and survival was compared between groups using the log-rank test. The effect of variables on OS or RFS was determined using univariate and multivariate analyses as well as the Cox proportional hazards model, using JMP software. The analyses classified pathologic TNM stages as I–II or III–IV.

3 | RESULTS

3.1 | Genomic aberration profiles of 74 patients with G3EEC

We summarized the clinical characteristics and pathological data of the 74 patients in Table 1 and Figure 1A. Among the patients, 49 had early-stage (Federation of Gynecology and Obstetrics [FIGO] stage I–II) and 25 had advanced-stage (FIGO stage III–IV) disease. None of the early-stage patients received adjuvant therapy. Here, 20 of the 25 patients with advanced-stage disease received platinum-containing regimens, including doxorubicin/cisplatin, paclitaxel/carboplatin, and cyclophosphamide/doxorubicin/cisplatin; two received radiation therapy; and the remaining three refused adjuvant therapy. In total, 261 oncogenic/pathogenic/nonsense mutations were detected in the 74 Japanese patients with G3EEC, and these included 138 nonsynonymous,

TABLE 1 Characteristics of patients with grade 3 endometrial endometrioid carcinoma

Variables	n (%)
Total patients	74
Age, median (range) [years]	57 (37–80)
FIGO stage (2008)	
I	44 (59.5)
II	5 (6.8)
III	18 (24.3)
IV	7 (9.5)
Depth of invasion	
Endometrium	9 (12.2)
Myometrium <50%	24 (32.4)
Myometrium ≥50%	41 (55.4)
Cervical stromal involvement	
Present	15 (20.3)
Absent	59 (79.7)
Lymphovascular space invasion	
Present	48 (64.9)
Absent	26 (35.1)
Pelvic cytology	
Positive	20 (27.0)
Negative	54 (73.0)

Abbreviation: FIGO, International Federation of Gynecology and Obstetrics.

59 stop-gain, 39 frame-shift, and 25 splicing-site mutations. _PTEN_, the most frequently mutated gene, was detected in 44/74 (59.5%) patients, followed by _ARID1A_, _TP53_, _PIK3CA_, and _POLE_ in 39/74 (52.7%), 36/74 (48.6%), 32/74 (43.2%), and 22/74 (29.7%) patients, respectively. Six of 22 patients with _POLE_ mutations had non-_POLE_ EDMs (Figure 1C). The _CTNNB1_ mutations were detected in 7/74 (9.5%) patients. Copy number amplifications in _MYC_ were detected in 8/74 (10.8%) patients, and those in _ERBB2_ were detected in 3/74 (4.0%) patients. No copy number amplifications were detected in _PIK3CA_ (Figure 1C).

Actionable genomic alterations were identified in patients with G3EEC in this study. Actionable genomic alterations registered as evidence levels 1–3 in OncoKB were detected in 53/74 (71.6%) patients with G3EEC (Figure S2). Thirty-eight (51.4%) patients were potential candidates for inhibition therapy targeting the PIK3CA/AKT/mammalian target of rapamycin (mTOR) pathways, and 25 (33.8%) patients may have received more than one molecular-targeted therapy.

3.2 | IHC profiles for MMR, p53, and ARID1A

The results of IHC staining for G3EEC are shown in Figure 1B. Loss of PMS2 and MSH6 protein expression was observed in 44.6% of
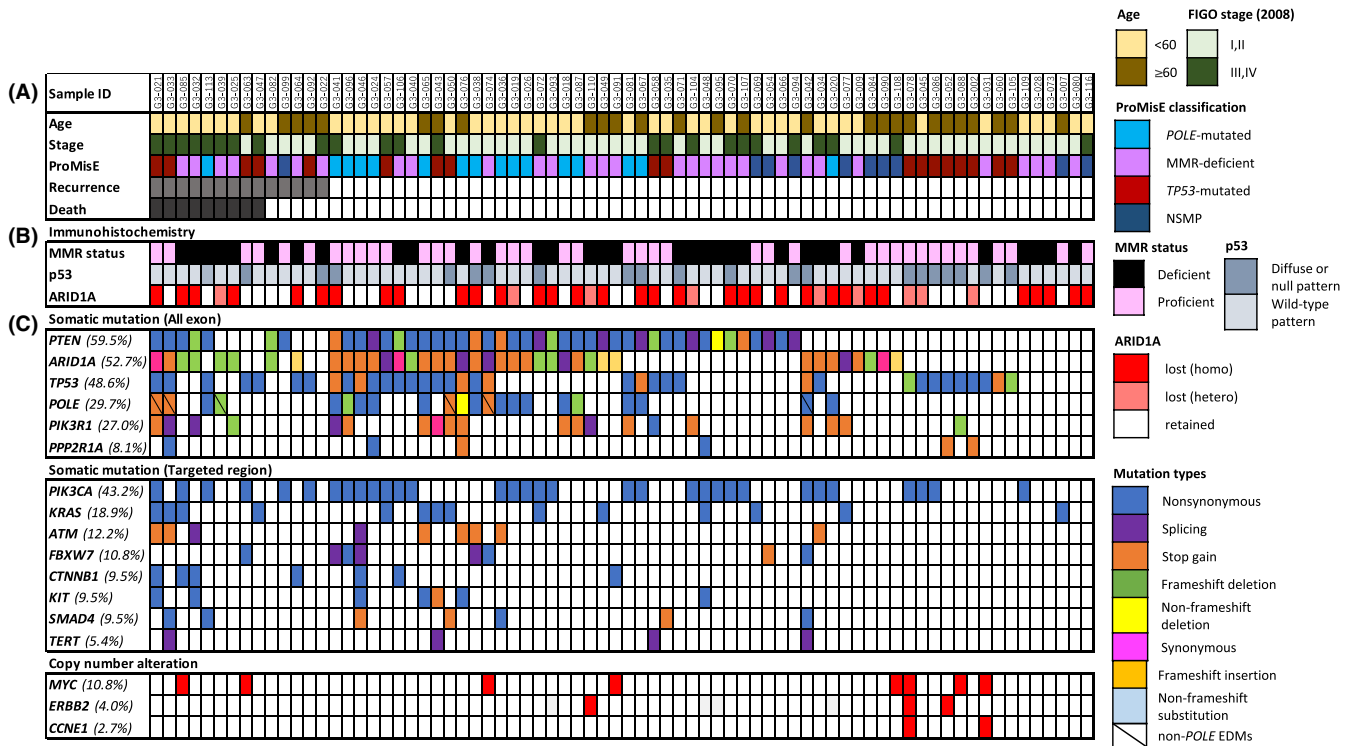
FIGURE 1 Detection of clinicopathological factors and a mutation profile greater than 5% in our cohort. (A) Clinical factors and recurrence status. (B) IHC staining. (C) Mutation profiles of the 74 patients with G3EEC. Mutated genes are color-coded according to their mutation type. Abbreviations: EDMs, exonuclease domain mutations; IHC, immunohistochemical; MMR, mismatch repair; NSMP, no specific molecular phenotype
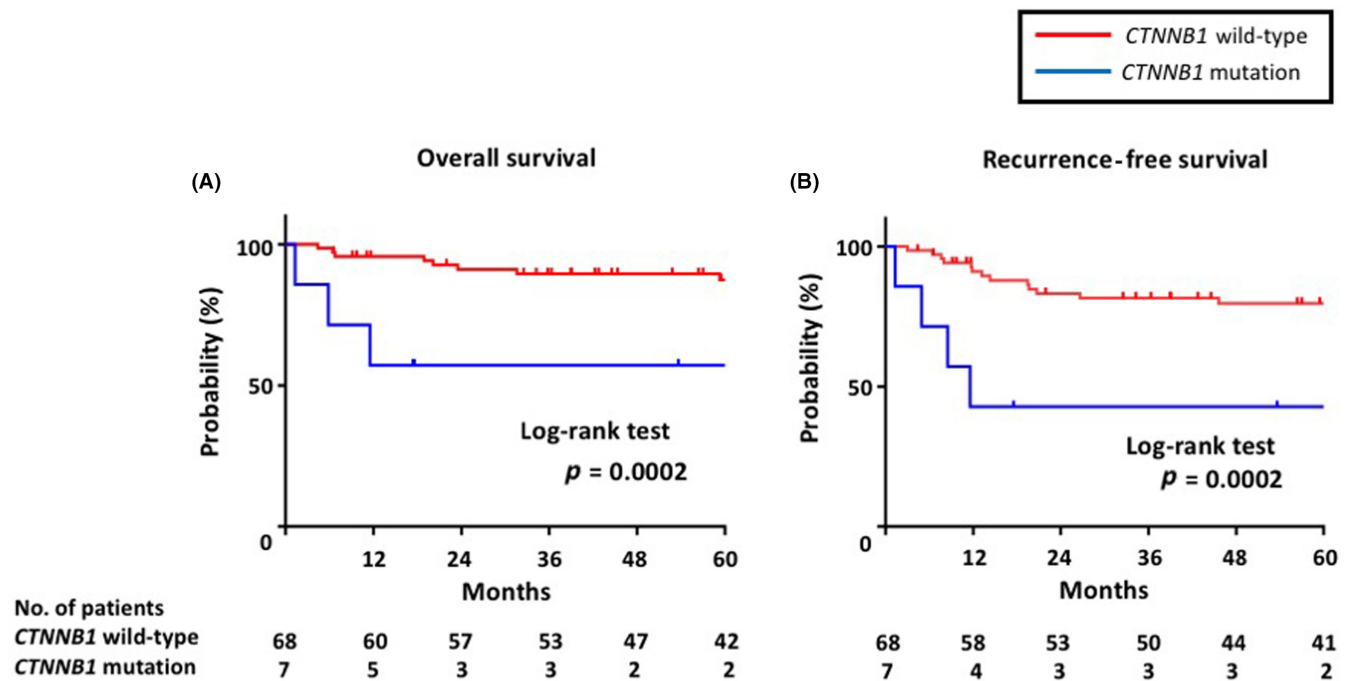

FIGURE 2 Kaplan–Meier survival curves according to the *CTNNB1* status in all stages. (A) Overall survival of *CTNNB1* wild-type (red line) and *CTNNB1* mutants (blue line). (B) Recurrence-free survival of *CTNNB1* wild-type (red line) and *CTNNB1* mutants (blue line)

the cases. Loss of ARID1A expression was observed in 56.8% of the patients. Loss of MMR protein expression was significantly associated with the loss of ARID1A expression ($p = 0.0004$). However, the expression statuses for MMR and of p53 and ARID1A were not correlated with the clinicopathological features.

3.3 | Correlation between *CTNNB1* genomic alterations and clinical outcomes in 74 patients with G3EEC

The median follow-up time of the surviving patients was 63 months (range 1–130 months). Significantly worse OS and RFS were observed in seven patients with *CTNNB1* genomic alterations than in patients without *CTNNB1* genomic alterations (log-rank test; $p = 0.0002$ and $p = 0.0002$, respectively) (Figure 2A,B). This tendency was similar to that observed in stages III and IV patients only (log-rank test; $p = 0.0006$ and $p = 0.02$, respectively) (Figure S3A,B). Four of the seven patients with *CTNNB1* genomic alterations showed distant metastasis (three patients had lung metastasis and one patient had bone metastasis) at the initial visit. Other genomic alterations were not significantly correlated with clinical outcomes. Previously reported prognostic factors, such as age [29] and pathological stage (I–II vs. III–IV), were used as adjustment factors during the Cox proportional hazards model analysis. The *CTNNB1* genomic alterations were correlated with worse OS (hazard ratio = 10.9; $p = 0.006$) (Table 2) and RFS (hazard ratio = 6.34; $p = 0.004$) (Table S1). The *CTNNB1* genomic alterations were identified as independent prognostic factors in our study. Moreover, in the IHC analysis, all samples with the *CTNNB1* mutation (seven cases) showed positive nuclear staining for CTNNB1, whereas the absence of *CTNNB1* mutation led to the lack of nuclear accumulation of CTNNB1 (Figure S6).

3.4 | Comparison of mutational patterns between Caucasian and Asian patients with G3EEC

We compared the genomic alteration profiles determined in our study with those registered in the dataset from TCGA. Alterations in *PTEN*, the most frequently mutated gene, were detected in 91/132 (68.9%) patients, and alterations in *PIK3CA*, *ARID1A*, and *TP53* were detected in 61/132 (46.2%), 61/132 (46.2%), and 40/132 (30.3%) patients, respectively (Figure 3). The frequencies of *POLE* EDMs and

TP53 genomic alterations determined in our study were higher than those in TCGA (Fisher's exact test; $p = 0.02$ vs $p = 0.01$, respectively; Table 3). Hotspot mutations in *POLE* primarily included the P286R point mutation (857C > G) and V411L point mutation (1231G > C/T).

3.5 | Correlation between clinical outcomes and ProMisE molecular subtypes in our cohort

We classified 74 patients in our cohort into four subgroups, namely the *POLE* mutation, MMR deficiency, *TP53* mutation, and NSMP groups, based on the ProMisE molecular subtypes. The *POLE* mutation and NSMP groups showed favorable prognoses with OS and RFS (Figure 4). However, the OS and RFS of the MMR-deficient and *TP53*-mutated groups were worse than those of the former two groups. The RFS of the *POLE* mutated group tended to be better than that of groups with *TP53* mutations and MMR deficiency ($p = 0.08$ vs $p = 0.07$, respectively). However, this association was not observed between the groups with *POLE* mutations and p53 abnormality (determined via IHC staining) ($p = 0.28$) (Figure S4). Three cases of non-*POLE* EDMs were classified in each of the *TP53*-mutated and MMR deficiency groups. The OS and RFS of non-*POLE* EDMs were significantly worse than those of *POLE* EDMs ($p = 0.01$ vs $p = 0.007$, respectively) (Figure S5). We examined whether the ProMisE molecular subtypes were associated with clinicopathological factors. The proportion of patients with cervical stromal invasion was higher in the groups with *POLE* mutations and NSMP than in those with MMR deficiency and *TP53* mutations ($p = 0.008$) (Table S2).

4 | DISCUSSION

In this study, we explored the genomic alteration profiles of Japanese patients with G3EEC and found that *CTNNB1* mutations were independently correlated with worse clinical outcomes. The frequencies of *POLE* and *TP53* genomic alterations were significantly higher in our cohort than in TCGA, which represents the Caucasian population. Moreover, the classification of ProMisE molecular subtypes tended to be associated with the prognoses of Japanese patients with G3EEC.

Notably, we newly found that *CTNNB1* genomic alterations can be a poor prognostic factor for patients with G3EEC. Several prior studies have reported that *CTNNB1* is significantly associated with

TABLE 2 Correlation between *CTNNB1* mutation and overall survival in grade 3 endometrial endometrioid carcinoma

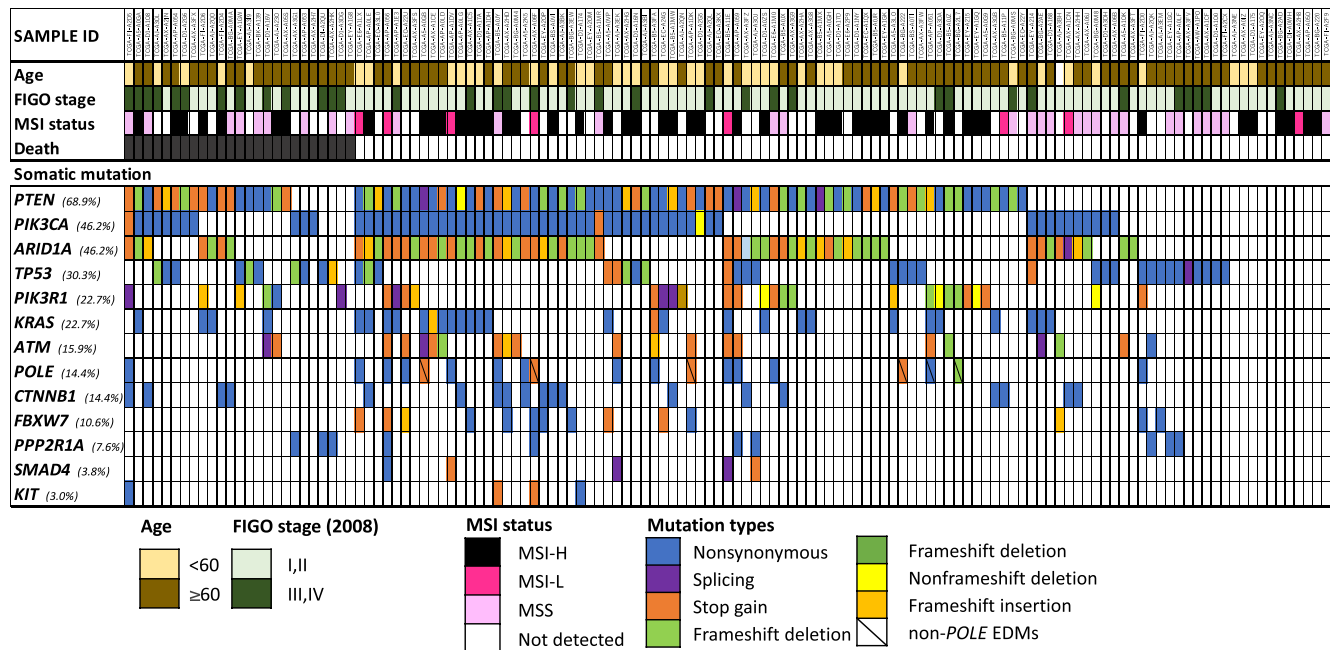| Variable	Univariate		Multivariate[a]	
	Hazard ratio (95%CI)	*p*-value	Hazard ratio (95%CI)	*p*-value
Age (<50/≥50)	4.74 (1.33–16.9)	0.016	1.07 (0.30–3.86)	0.91
Stage (III, IV/I, II)	21.1 (2.66–167)	0.004	30.7 (3.64–258)	0.002
CTNNB1 mutation (n = 7)	8.81 (2.12–36.6)	0.003	10.9 (2.01–58.7)	0.006

[a]Stepwise Cox regression analysis.

FIGURE 3 Clinicopathological factors and mutation profiles in The Cancer Genome Atlas dataset. Abbreviations: EDM, exonuclease domain mutation; MSI, microsatellite instability; MSS, microsatellite stability

TABLE 3 Comparison of mutation patterns between Caucasians and Asians with grade 3 endometrial endometrioid carcinoma

Variable	Our study (n = 74)	TCGA database (n = 132)	p-value[a]
Age			
Median (range) [year]	57 (37–80)	64 (53–90)	<0.01
PTEN mutation	44 (59.5%)	91 (68.9%)	0.17
ARID1A mutation	39 (52.7%)	61 (46.2%)	0.45
TP53 mutation	36 (48.6%)	40 (30.3%)	0.01
PIK3CA mutation	32 (43.2%)	61 (46.2%)	0.79
PIK3R1 mutation	20 (27.0%)	30 (22.7%)	0.60
POLE mutation[b]	16 (21.6%)	13 (9.8%)	0.02
KRAS mutation	14 (18.9%)	30 (22.7%)	0.64
CTNNB1 mutation	7 (9.5%)	19 (14.4%)	0.42
PPP2R1A mutation	6 (8.1%)	10 (7.6%)	1.00

[a]Fisher's exact test.
[b]POLE exonuclease domain mutations only.

worse RFS in the context of low-grade, early-stage endometrioid carcinoma.[32,33] The Translational Research in Post-Operative Radiation Therapy in the EC molecular classification system, which incorporates CTNNB1 sequencing, considers the CTNNB1 mutation group as an intermediate risk, which is similar to the above findings.[17] Our study suggested that the clinical impact of CTNNB1 mutants was applicable not only in patients with low-grade and early-stage tumors but also in patients with G3EEC.

Normal Wnt/β-catenin signaling is essential for maintaining stem cells. However, exon 3 deletion in CTNNB1 in a murine model caused the upregulation of the Wnt/β-catenin pathway, and its constitutive activation caused the generation of tumor-initiating or cancer stem cells.[32] Cancer stem cells develop into endometrial hyperplasia, and eventually into invasive endometrioid-type endometrial carcinoma, in response to the sequential accumulation of other genetic alterations, including those in PTEN.[34,35] Traf2- and Nck-interacting kinase (TNIK), an essential component of the T-cell factor-4 and β-catenin transcriptional complexes, regulates Wnt signaling in the most downstream part of the pathway. In 2016, the orally available small-molecule TNIK inhibitor, NCB-0846, which exhibits anti-Wnt activity, was first reported in Japan.[36] Its pharmacological inhibition is expected to block signaling with CTNNB1 mutations.

Although it has been reported that POLE mutations in G3EEC are associated with a lower risk of recurrence and death,[35] this association was not significant in the current study. The group with mutations in POLE EDMs in the ProMisE classification showed favorable prognoses with respect to the OS and RFS in our cohort. Furthermore, patients with POLE EDMs exhibited significantly better OS and RFS than patients with non-POLE EDMs, a finding consistent with the findings of previous studies.[30] These results indicated that the POLE EDM status reflects the need for a change in the treatment of Japanese patients with G3EEC, for which the exclusion of adjuvant therapy and decreased surveillance may be appropriate.

We compared the mutational profiles of Caucasian and Japanese patients with G3EEC. The somatic mutation profile of Japanese patients with G3EEC was similar to that of Caucasian patients, with a few exceptions.[7] PTEN, ARID1A, and PIK3CA mutations were frequent in both Caucasian and Japanese patients with G3EEC, and this result was consistent with the results of previous studies.[6,12,37] In contrast, the frequency of POLE EDM genomic alterations in our cohort was approximately twice as high as that reported in TCGA.
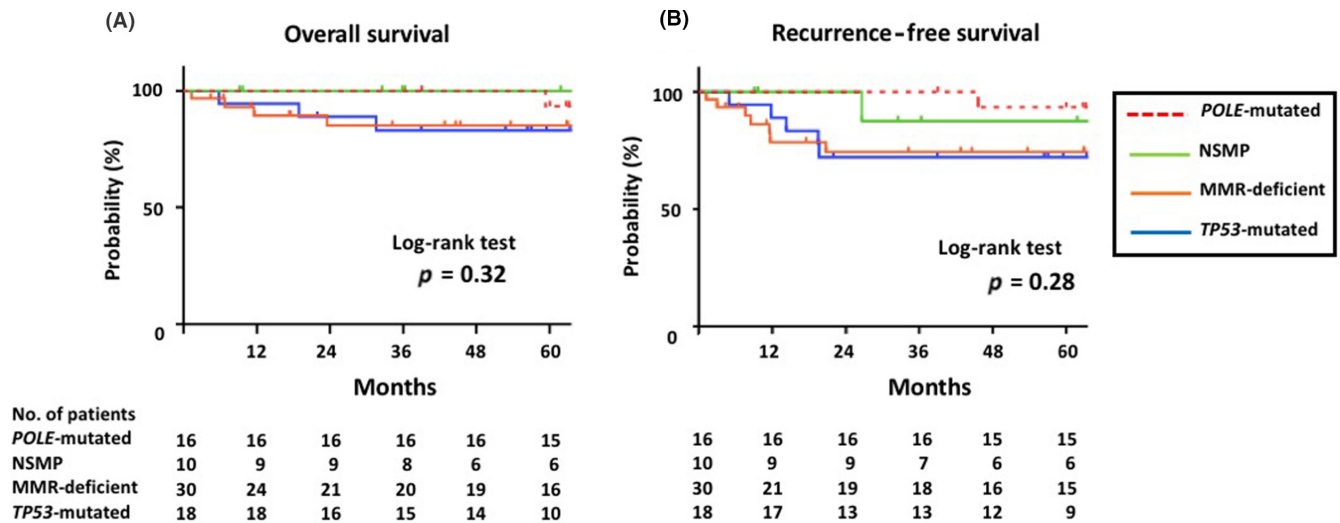
FIGURE 4 Kaplan–Meier survival curves according to molecular subtypes. (A) Overall survival and (B) Recurrence-free survival. Next-generation sequencing was used to categorize the *TP53* status into two groups: *TP53* wild-type and *TP53* mutant. Abbreviations: MMR, mismatch repair; NSMP, no specific molecular phenotype

A previous study demonstrated that the 5-year OS in patients with early-stage G3EEC was 96.4% in the absence of adjuvant therapy, whereas that in patients with stage III G3EEC was 85.6% with adjuvant chemotherapy.[21] One possible explanation for the favorable prognosis is that cases with mutations in *POLE* EDMs, corresponding to stage I in our cohort, were more frequent in this study cohort (Table S2). The above findings indicate that treatment strategies applied to Caucasian patients with EECs may also be applied to Japanese patients with G3EEC.

Using the *TP53* mutation instead of p53 expression as a marker, we showed that prognoses were associated with the ProMisE molecular groups. In this study, we frequently observed patients with the co-occurrence of a p53 wild-type pattern identified using IHC and a *TP53* mutation. The intratumoral heterogeneity of the *TP53* mutation may explain this discrepancy. Unlike serous carcinomas derived from precursors with *TP53* mutations,[38] most G3EECs with *TP53* mutations evolve clonally from EIN/low-grade EECs without *TP53* mutations.[38] Intratumoral heterogeneity for the *TP53* mutation in G3EEC results from this clonal evolution. An abnormal p53 staining pattern was defined as the presence of strong positivity with diffuse expression or a complete lack of p53 expression. However, a subclone with *TP53* mutations was frequently encountered in patients with G3EEC having focal abnormal p53 staining patterns. In such cases, DNA sequencing can detect more than 4% of tumor cells with *TP53* mutations, which cannot be identified using IHC staining. Furthermore, endometrial carcinomas display subtypes with a high mutational burden that can result in secondary or passenger *TP53* driver mutations that lack the same biological significance as *TP53* driver mutations in serous carcinomas,[38] the expression patterns of which have not been studied well. Further studies to explore the characteristics of EEC with discordant p53 IHC results and *TP53* mutation status are needed.

Moreover, patients with deficient MMR exhibited a high frequency of ARID1A expression loss in our cohort. This is consistent with the findings of a study pertaining to colorectal carcinoma, gastric carcinoma, endometrial carcinoma, and ovarian endometrioid carcinoma.[39] However, in our cohort, we did not investigate the association between the loss of ARID1A and the prevalence of sporadic MSI (MLH1 promoter hypermethylation), as reported by previous studies.[39,40] In addition, no association between the loss of ARID1A and clinicopathological factors was found. Further studies may be needed to elucidate the factors leading to the co-occurrence of ARID1A inactivation and deficient MMR in G3EEC.

This study has some limitations. First, it was a single-center study with a retrospective design. Second, only seven cases (9%) with *CTNNB1* mutations were analyzed in this study. The contribution of *CTNNB1* mutations to the prediction of unfavorable prognosis might be relatively minor. Further studies to validate the effects on *CTNNB1* variants are needed in the near future.

In conclusion, we analyzed the mutation spectrum of Japanese patients with G3EEC, which exhibits frequent genomic alterations in *PTEN* (59.5%), *ARID1A* (52.7%), *TP53* (48.6%), *PIK3CA* (43.2%), and *POLE* (29.7%). Our results indicated the prognostic value of *CTNNB1* mutations, and the prognosis tended to be associated with that of the ProMisE molecular classification. Therefore, mutational patterns comparing Caucasian and Japanese patients with G3EEC were similar, although the frequency of *POLE* mutations was higher in Japanese patients. Patients with G3EEC may benefit from similar molecular-targeted therapies, regardless of ethnicity. Additionally, the *POLE* mutation status may indicate the need for changes in the treatment of Japanese patients with G3EEC, with the omission of adjuvant therapy.

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DISCLOSURE

The authors state that there are no conflicts of interest to declare.

ETHICAL APPROVAL

All patients provided written informed consent. The Institutional Review Board of the National Cancer Center Research Institute approved this study.

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