Endometrial stromal tumor is an uncommon uterine tumor comprising < 10% of all uterine mesenchymal neoplasms. The diagnosis of low-grade endometrial stromal sarcoma (LGESS) is based on characteristic histopathological features showing diffuse growth of uniform stromal cells resembling those of proliferative phase endometrium and uniformly distributed small spiral arterioles and it is not problematic in most LGESS cases. However, it is often difficult when the tumor displays diverse histological differentiation. A variety of histological differentiation types, including smooth muscle cells, fibromyxoid tissue, ovarian sex cord-like/epithelioid features, endometrioid glandular structures, atypical bizarre nuclei, rhabdoid or clear cell phenotype, adipose tissue or skeletal muscle, and predominant cystic changes have been described in the literature. When differentiation occurs, it frequently obscures the characteristic features of stromal cells and vascular pattern of LGESS, causing diagnostic difficulties.

In contrast, undifferentiated endometrial sarcoma (UES) does not histologically exhibit endometrial stromal differentiation, thus, the origin of UES can be controversial, however, combined LGESS and UES cases clearly suggest that at least a subset of UES is of endometrial stromal origin. The diagnosis of UES should only be made after excluding histologically similar tumors including poorly differentiated carcinoma, leiomyosarcoma, carcinosarcoma, and other specific sarcomas of the uterus by adequate sampling and immunohistochemical staining.

Various ancillary techniques have been employed to facilitate the diagnosis of LGESS, including immunohistochemical staining for estrogen receptor (ER) and progesterone receptor (PR), smooth muscle actin (SMA), β-catenin, and h-caldesmon. However, their value in the differential diagnosis has not been determined.

\( \text{JAZF1/JJAZ1} \), derived from the fusion of two zinc-finger genes (\( \text{JAZF1} \) located at chromosome 7p15 and \( \text{JJAZ1} \) at 17q21),
has been described as a characteristic feature of LGESS. However, reports vary on its prevalence in LGESS, ranging from 23% to 100%, and its diagnostic utility in ESS cases showing various types of histological differentiation and in UES remains to be established.

In this study, we analyzed the prevalence of the JAZF1/JJAZ1 fusion gene in 28 cases of endometrial stromal sarcoma including classical LGESS and those with various types of secondary histological differentiation, UES, and their histological variants to determine its applicability in the diagnosis of endometrial stromal sarcomas.

**MATERIALS AND METHODS**

**Case collection**

We retrieved all cases of endometrial stromal sarcoma diagnosed in the Department of Pathology, Asan Medical Center, Seoul, South Korea, from September 2003 to December 2009. Hematoxylin and eosin-stained slides of 38 cases were available for review by two pathologists (SRL, KRK), and formalin-fixed and paraffin-embedded tissue samples were available in 28 cases. Diagnoses of LGESS and UES were made according to the definition in the 2003 World Health Organization (WHO) Classification System. The study cases included 15 tumors with classical features of LGESS and six cases of LGESS with secondary histological differentiation, including smooth muscle differentiation (n=3), sex cord-like differentiation (n=2), and osseous differentiation (n=1), five UES, one UES with sex cord-like differentiation, and one case composed of combined LGESS and UES.

Among the 21 cases of LGESS and seven cases of UES (including histological variants), 21 cases (15 LGESS and six UES) occurred in the uterus. The tumors in the remaining seven patients involved extrauterine sites, including the pelvic cavity, peritoneum, and colon, but all seven patients had received a hysterectomy at an outside hospital due to uterine masses. These seven tumors in extrauterine sites consisted of classical LGESS in five cases, LGESS with osseous differentiation in one, and UES with sex cord-like differentiation in one case.

**Review of histological findings and immunohistochemical staining**

Diagnosis of the classic type of LGESS was based on the characteristic histopathological features of uniform stromal cells resembling those of proliferative phase endometrium and uniformly distributed small spiral arterioles. Six cases showing secondary histological differentiation had typical histological features of LGESS in some part of the tumor, and diagnoses were based on the histological and immunohistochemical features.

All cases were immunostained with antibodies for the ER (1:50, clone 6F11, monoclonal, Novo, Newcastle, UK), PR (1:200, clone 312, Novo), CD10 (1:25, clone 56C6, Novo), SMA (1:400, clone 1A4, Dako, Glostrup, Denmark), S-100 (1:200, polyclonal, Zymed, San Francisco, CA, USA), desmin (1:200, clone D33, Dako), vimentin (1:250, clone V9, Zymed), myogenin (1:200, Neomarkers, Fremont, CA, USA), cytokeratin (CK; 1:400, clone AE1/AE3, Zymed), and inhibin-α (1:100, clone R1, Serotec, Munich, Germany). UES was diagnosed based on the microscopic features of high-grade sarcoma with absence of significant epithelial, smooth muscle, skeletal muscle, or neural differentiation defined by immunonegativity for CK, SMA, desmin, myogenin, or S-100, respectively.

**Nested RT-PCR for constructing the JAZF1/JJAZ1 fusion gene**

Formalin-fixed and paraffin-embedded tissue were used for nested reverse transcription polymerase chain reaction (RT-PCR) in 28 cases, which included classical LGESS (n=15) and LGESS with smooth muscle differentiation (n=3), sex cord-like differentiation (n=2), osseous differentiation (n=1), UES (n=5), UES with sex cord-like differentiation (n=1), and combined LGESS and UES (n=1). Normal endometrium in the proliferative (n=2) and secretory (n=1) phases and tissue from endometriotic foci (n=3) in the posterior cul-de-sac were included for comparison. Tissues obtained from the differentiated areas were used for molecular analysis of tumors displaying secondary histological differentiation. In cases of LGESS with smooth muscle differentiation, each area showing different histological features was examined separately, and a sample including both components was used in cases of combined LGESS and UES.

Sections (6 μm-thick) of formalin-fixed, paraffin-embedded tissue were deparaffinized and incubated with 250 μL RNA digestion buffer (20 mM Tris, pH 7.6, 20 mM ethylenediaminetetraacetic acid, 1% sodium dodecyl sulfate) plus 5 μL proteinase K (20 mg/mL) at 56°C overnight. RNA was extracted with 750 μL of TRIzol (Invitrogen Life Technologies, Los Angeles, CA, USA) and incubated at room temperature for 10 minutes. Tissue pellets were washed with 75% ethanol after chloroform
DNA sequencing of the JAZF1/JJAZ1 fusion gene

Amplified bands representing the JAZF1/JJAZ1 fusion transcripts of two randomly selected positive cases were sequenced to verify the PCR product sequences. Sequencing reactions were performed with 1 µL of treated PCR product and exonuclease I, 0.5 µL of BigDye Ready Reaction Mix (ABI PRISM BigDye Terminator v3.1, Applied Biosystems, Foster City, CA, USA), 1.5 µL of 5 × sequencing buffer (ABI PRISM BigDye Terminator v3.1, Applied Biosystems), 1 µL of primer (1 pmol/µL) and 6 µL double-distilled H₂O. Sequencing conditions comprised 25 cycles of initial denaturation at 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes. For purification, 10 µL of sequencing products were incubated with 1 µL of 3M sodium acetate (pH 4.6, Bioneer Co., Daejeon, Korea) at room temperature for 10 minutes and centrifuged at 13,000 rpm for 30 minutes at 25°C. After centrifugation, pellets were washed with 200 µL of 70% ethanol and air-dried. Purified sequencing products were dissolved in 10 µL of Hi-Di™ formamide (Applied Biosystems) as an injection solvent. Products were sequenced using the ABI PRISM DNA Analyzer 3100 Automatic DNA Sequencer (Applied Biosystems), and the sequences were analyzed and compared using Sequencer 3.1.1. software (Applied Biosystems).

RESULTS

Histopathological and immunohistochemical features

Among seven extrauterine cases, previous uterine masses were diagnosed as leiomyomas in two cases. A review of the histological features in one case revealed a smaller portion of LGESS features in addition to leiomyoma-like area in the majority of the tumor. The histological findings of the other case were not available for review, but multiple vascular tumor emboli were identified at the extraterine site, suggesting the possibility of metastasis from other sites. Uterine masses of the remaining five cases were diagnosed as classical LGESS in three cases, combined LGESS and UES in one, and ESS in one case.

Two cases of LGESS with smooth muscle differentiation displayed well-circumscribed tumor margins at low magnifications which were similar to those of leiomyoma. The inner portion showed characteristic microscopic features of LGESS, but the outer portion was mostly composed of benign appearing smooth muscle bundles identical to those of typical leiomyoma (Fig. 1A). The cellularity of the outer leiomyoma-like area was as low as that of typical benign leiomyoma, and neither mitotic figures nor cytologic atypia were identified. Because of a well-circumscribed external margin of the tumor, it was difficult to distinguish an endometrial stromal nodule showing smooth muscle differentiation from a cellular leiomyoma. In the third case, showing smooth muscle differentiation, some discrete leiomyomatous nodules were observed within the classic LGESS area (Fig. 1B). The leiomyomatous area showed strong SMA and CD10 immunopositivity. The nodular leiomyomatous areas had thick-walled blood vessels instead of the small arterioles seen in LGESS.

In the LGESS cases with ovarian sex cord-like differentiation, anastomosing trabeculae composed of epithelioid cells resembled those of a granulosa cell tumor of the ovary (Fig. 1C). The sex cord-like area showed CD10, ER, and PR immunopositivity. The case with osteoid differentiation showed amorphous eosinophilic osteoid material with woven bone formation within solid sheets of spindle cells (Fig. 1D). The characteristic vascu-
lar pattern of LGESS was lost in that area; however, the presence of classic LGESS features in other areas made it possible to provide a diagnosis.

UES cases showed diffuse or fascicular arrangement of poorly differentiated spindle cells with varying degrees of nuclear pleomorphism and frequent mitotic activity ranging from 6 to 48 mitoses per 10 high-power fields (Fig. 1E). The characteristic vascular pattern was not observed. The tumor cells showed diffuse positivity for CD 10 in all seven cases, and two cases showed strong ER and PR immunopositivity.

One case of UES contained trabecular or cord-like structures composed of epithelioid cells containing vesicular nuclei with a scanty amount of eosinophilic cytoplasm in a background of eosinophilic or myxoid stroma (Fig. 1F). The tumor cells were strongly positive for ER, PR, vimentin, and CD10, but negative for CK, SMA, S-100, myogenin, and inhibin-α, suggesting endometrial stromal differentiation.

In the case of combined LGESS and UES, the tumor was di-
vided into three nodular areas showing different histological features (Fig. 1G). The inner portion of the mass showed undifferentiated spindle cells with significant hypercellularity and increased mitotic figures up to 47 mitoses per 10 high power fields and myxoid changes in the stroma (Fig. 1H). Classical features of LGESS were retained in the midportion of the mass, whereas the outer portion showed smooth muscle differentiation resembling a leiomyoma (Fig. 1I).

Analysis of JAZF1/JJAZ1 gene fusion

The JAZF1/JJAZ1 fusion transcript was detected in 18 (85.7%) of 21 LGESS cases (Fig. 2A). Positive LGESS cases included 14 cases with classic features of LGESS, one with smooth muscle differentiation (33%), two with sex cord-like differentiation (100%), and one with osseous differentiation (100%). In the LGESS case showing smooth muscle differentiation, the JAZF1/JJAZ1 fusion transcript was detected only in the classical area, but the smooth muscle differentiated area did not reveal the fusion transcript.

Three samples of normal endometrium (two proliferative and one secretory phases) and three cases of endometriosis occurring in the posterior cul-de-sac did not show the fusion transcript (Fig. 2B).

In contrast to LGESS, JAZF1/JJAZ1 transcripts were detected in two (28.6%) of seven UES cases (Fig. 3). The case of UES with sex cord-like differentiation and the case of combined LGESS and UES did not show the fusion transcript (Table 1).

The JAZF1/JJAZ1 transcripts were detected in 13 (61.9%) of 21 cases that occurred in the uterus, whereas the fusion transcripts were detected in all seven (100%) extrauterine cases.

Direct DNA sequencing

Breakpoints were identical between the two cases of LGESS (Fig. 4). The detected breakpoint, t(7;17)(p15;q21), was identical to that described previously.15 The base sequence resulting from the translocation created a new glutamic acid residue.

DISCUSSION

A variety of histological differentiation types frequently obscure the characteristic features of stromal cells and vascular pattern of LGESS, causing diagnostic difficulties. This study began with a few difficult cases in our routine practice. When an LGESS shows significant smooth muscle differentiation throughout the tumor, individual cells closely resemble those of leiomyoma and the margin is often well circumscribed from the surrounding myometrium. Mitotic figures and characteristic

Table 1. Summary of nested reverse transcription polymerase chain reaction results for the JAZF1/JJAZ1 fusion gene in 28 endometrial stromal sarcoma (ESS) cases

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
<th>No. of JAZF1/JJAZ1 fusion gene positive cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGESS, classic</td>
<td>15</td>
<td>14 (93.3)</td>
</tr>
<tr>
<td>LGESS with smooth muscle differentiation†</td>
<td>3</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>LGESS with sex cord-like differentiation</td>
<td>2</td>
<td>2 (100)</td>
</tr>
<tr>
<td>LGESS with osseous differentiation</td>
<td>1</td>
<td>1 (100)</td>
</tr>
<tr>
<td>UES, not otherwise specified</td>
<td>5</td>
<td>2 (40)</td>
</tr>
<tr>
<td>UES with sex cord-like differentiation</td>
<td>1</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Combined LGESS and UES</td>
<td>1</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

†Fusion gene is identified only in the classical area of LGESS.
LGESS, low-grade ESS; UES, undifferentiated endometrial sarcoma.
LGESS vascular patterns disappear along the cellular differentiation. Thus, histological findings are almost indistinguishable from those of leiomyoma or cellular leiomyoma. Even when the LGESS had partial smooth muscle differentiation at the periphery, it created a well circumscribed margin from the surrounding normal myometrium as in cases of leiomyoma; thus, histologically distinguishing this from an endometrial stromal nodule with smooth muscle differentiation is often difficult, which was formerly designated as “stromomyoma.” The absence of distinct thick-walled blood vessels and cleft-like spaces can facilitate the diagnosis of ESS; however, these features were not observed in our cases. In the literature, five cases of LGESS showing secondary histological differentiation have been tested with the \( \text{JAZF1/JJAZ1} \) fusion gene,\(^{20,24} \) but only two cases with secondary histological differentiation showed \( \text{JAZF1/JJAZ1} \) gene fusion, which were shown in tumors with sex cord-like differentiation and fibromyxoid differentiation, respectively. LGESS cases with smooth muscle differentiation in the literature did not show the fusion gene as in our cases. Therefore, the diagnosis of LGESS with smooth muscle differentiation in the literature did not share the fusion gene as in our cases. Therefore, the diagnosis of LGESS with smooth muscle differentiation should be based on histological and immunohistochemical findings, and an examination for the \( \text{JAZF1/JJAZ1} \) fusion gene does not seem to be helpful in the diagnosis.

The differential diagnosis of LGESS with endometrioid glandular differentiation from extensive intestinal endometriosis is also problematic. In cases of extensive endometriosis, lymph node and intravascular involvement can sometimes be evident, which often misleads the diagnosis to LGESS. Immunohistochemical staining for Ki-67, ER, PR, CD10, and cyclin D1 are not helpful in the differential diagnosis. Recently, immunohistochemical expression of nuclear \( \beta \)-catenin has been suggested as a useful ancillary technique for the differential diagnosis of LGESS from normal endometrial stroma and cellular leiomyoma.\(^ {19,29} \) However, we could not confirm a difference in nuclear \( \beta \)-catenin expression in typical cases of endometriosis and LG-ESS. We experienced two such cases in which endometrioid-like tissue involved the intestinal wall, pericolic lymph nodes, and vascular spaces. The differential diagnosis between extensive intestinal endometriosis and LGESS showing endometrioid glandular differentiation was extremely difficult, but negativity for the \( \text{JAZF1/JJAZ1} \) gene fusion in conjunction with other histopathological and immunohistochemical features led us towards endometriosis.

The diagnosis of UES is usually made on an exclusion basis, as it has many overlapping histological features with other types of high-grade uterine sarcomas. Detection rates of \( \text{JAZF1/JJAZ1} \) fusion in UES cases vary from 0% to 50%\(^ {20,23,24} \). In our study, the fusion gene was detected in two (28.6%) of the seven UES cases. As the fusion gene has not been described in other types of uterine sarcomas, positive results in high-grade sarcomas may facilitate classification of UES, despite the low incidence.

Fig. 4. Sequencing results for the \( \text{JAZF1/JJAZ1} \) fusion transcript in two selected cases of low-grade endometrial stromal sarcoma. The detected breakpoint, t(7;17)(p15;q21), is identical to that in cases reported in the literature.
LGESS cases (67%) showing histological differentiation were also positive for JAZF1/JJAZ1 gene fusion, suggesting that the test can be helpful, if not conclusive, in difficult cases with histological differentiation, although the utility of the test needs to be confirmed in large-scale studies with more cases showing histological differentiation.

In our study, a difference was observed in the prevalence of the JAZF1/JJAZ1 fusion transcript according to the sites of tumor involvement, uterine vs extrauterine. However, it seemed to be due to inclusion of more UES cases with uterine tumors, rather than a true difference according to the site of tumor involvement.

We selected two fusion gene-positive LGESS cases for direct sequencing of PCR products. In both cases, the same breakpoint in the JAZF1/JJAZ1 fusion sequence, t(7;17)(p15;q21), was detected, identical to that previously described by Koontz et al.5 JAZF1 and JJAZ1 genes contain sequences encoding zinc-finger motifs resembling the majority of chromosomal translocations in soft tissue sarcomas. Rearrangement of JAZF1 results in the expression of a tumor-specific mRNA transcript containing 5′ JAZF1 and 3′ JJAZ1 sequences retaining zinc-finger motifs from each gene. The role of the JAZF1/JJAZ1 fusion protein is unclear at present. However, considering that wild-type JAZF1 is expressed in normal endometrium, the JAZF1/JJAZ1 fusion is likely to create a chimeric protein that disrupts transcription in a lineage-specific manner.10

In conclusion, the JAZF1/JJAZ1 fusion gene may be a helpful ancillary technique in difficult cases to confirm the diagnosis of endometrial stromal sarcoma, because the fusion transcript is identified in a significantly high proportion of patients with LGESS and secondary histological differentiation, except in those with smooth muscle differentiation. As this fusion gene is absent in other types of uterine sarcomas, positive results for JAZF1/JJAZ1 in high-grade uterine sarcomas may aid in their classification as UES.

REFERENCES

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