*The Korean Journal of Pathology 2011; 45: 498-505*
*http://dx.doi.org/10.4132/KoreanJPathol.2011.45.5.498*

# Diagnostic Utility of the *JAZF1/JJAZ1* Gene Fusion in Endometrial Stromal Sarcomas and Their Histologic Variants

Sang-Ryung Lee · Joon Seon Song
Ga-Hye Kim[1] · Jene Choi
Hyung Kyoung Kim · Yonghee Lee
Kyu-Rae Kim

Department of Pathology, Asan Medical Center, [1]Institute for Life and Science, University of Ulsan College of Medicine, Seoul, Korea

**Received:** July 12, 2011
**Accepted:** September 27, 2011

**Corresponding Author**
Kyu-Rae Kim, M.D.
Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, 388-1 Pungnap 2-dong, Songpa-gu, Seoul 138-736, Korea
Tel: +82-2-3010-4514
Fax: +82-2-472-7898
E-mail: krkim@amc.seoul.kr

*This work was presented as a poster at the 2011 annual meeting of the USCAP in San Antonio, U.S.A. This study was supported by a NRF (No. 2011-0026130) and a grant from the Asan Institute for Life Sciences (2011-230).

**Background:** The diagnosis of endometrial stromal sarcoma (ESS) is often difficult in cases showing diverse histological differentiation or in undifferentiated endometrial sarcoma (UES). Recently, *JAZF1/JJAZ1* gene fusion has been described as a defining feature of low-grade ESS (LGESS). However, its prevalence is variably reported, and the diagnostic utility has rarely been examined for cases showing various histological differentiation. **Methods:** To test the diagnostic utility of *JAZF1/JJAZ1* gene fusion in difficult cases, we compared the prevalence of the *JAZF1/JJAZ1* fusion gene in LGESS with and without histological differentiation. **Results:** The *JAZF1/JJAZ1* fusion transcript was detected in 18 of 21 LGESS (85.7%), including 14 classical LGESS (93%), four LGESS with diverse histological differentiation (67%), and two with UES (28.6%). Positive cases included two LGESS with sex cord-like differentiation, one with osseous differentiation, and two UES. LGESS showing smooth muscle differentiation revealed the fusion transcript only in the classic area. Direct sequencing analysis of two LGESS revealed a previously reported breakpoint at t(7;17)(p15;q21). **Conclusions:** The *JAZF1/JJAZ1* fusion gene was identified in a significant proportion of LGESS showing secondary histological differentiation except in cases with smooth muscle differentiation. Thus, this fusion gene may be useful to confirm the diagnosis in difficult cases of LGESS.

**Key Words:** Endometrial stromal sarcoma; *JAZF1/JJAZ1*; Gene fusion; Histologic differentiation; Undifferentiated endometrial sarcoma

Endometrial stromal tumor is an uncommon uterine tumor comprising < 10% of all uterine mesenchymal neoplasms.[1] 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.[2] However, it is often difficult when the tumor displays diverse histological differentiation. A variety of histological differentiation types, including smooth muscle cells,[3-5] fibromyxoid tissue,[3,4,6] ovarian sex cord-like/epithelioid features,[4] endometrioid glandular structures,[4,7,8] atypical bizarre nuclei,[9,10] rhabdoid or clear cell phenotype,[11-14] adipose tissue or skeletal muscle,[15] and predominant cystic changes[16,17] 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.[2] 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.[11,18-22] However, their value in the differential diagnosis has not been determined.

*JAZF1/JJAZ1*, derived from the fusion of two zinc-finger genes (*JAZF1* located at chromosome 7p15 and *JJAZ1* at 17q21),

has been described as a characteristic feature of LGESS.[23-25] However, reports vary on its prevalence in LGESS, ranging from 23% to 100%,[20,23-27] 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.[28] 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, Freemont, 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

extraction and precipitation with isopropanol. Samples were dried at room temperature and dissolved in RNase-free water. Extracted RNA was treated with DNase I at 37℃ for 30 minutes. Reactions were performed in a final volume of 20 μL using 4 μL of 5× buffer, 1 μL of 2.5 mM dNTP, 2 μL of random hexamer, 40 U/μL of RNase inhibitor, 200 U/μL of reverse transcriptase, and 1 μg of DNase-treated RNA. The reaction conditions were as follows: 65℃, 5 minutes; 42℃, 60 minutes; 70℃, 15 minutes. Amplification of *JAZF1/JJAZ1* was performed in a final reaction volume of 25 μL containing 2 μL of cDNA or the first PCR product, 2.5 μL of 10× buffer, 2 μL of dNTP, 1 μL each of sense and antisense primer, and 0.1 μL of *Taq* DNA polymerase. The primers used were *JAZF1*-outer forward: 5′-CACGCCACAGCAGTGGAAGC-3′, *JJAZ1*-outer reverse: 5′-TGTTTGTTCTGGAGTTTCGATGAGACA-3′, *JAZF1*-inner forward: 5′-AGCAGTGGAAGCCTTACTCC-3′, and *JJAZ1*-inner reverse: 5′-GACATGTAAGTAAGAGTTCTGTGCAA-3′. The following thermal cycling profiles were employed: an initial PCR activation step at 94℃ for 5 minutes, 35 cycles of denaturation at 94℃ for 40 seconds, annealing at 55℃ in the first PCR reaction and 60℃ in the second PCR reaction for 40 seconds, extension at 72℃ for 1 minute, and a final extension step at 71℃ for 10 minutes. Amplified products were 158 bp in length and visualized by electrophoresis on an agarose gel. RT-PCR of β-globin or glyceraldehyde 3-phosphate dehydrogenase was performed to assess RNA quality.

### 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 $H_2O$. Sequencing conditions comprised 25 cycles of initial denaturation at 96℃ for 10 seconds, 50℃ for 5 seconds, and 60℃ 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℃. 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 extrauterine 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-

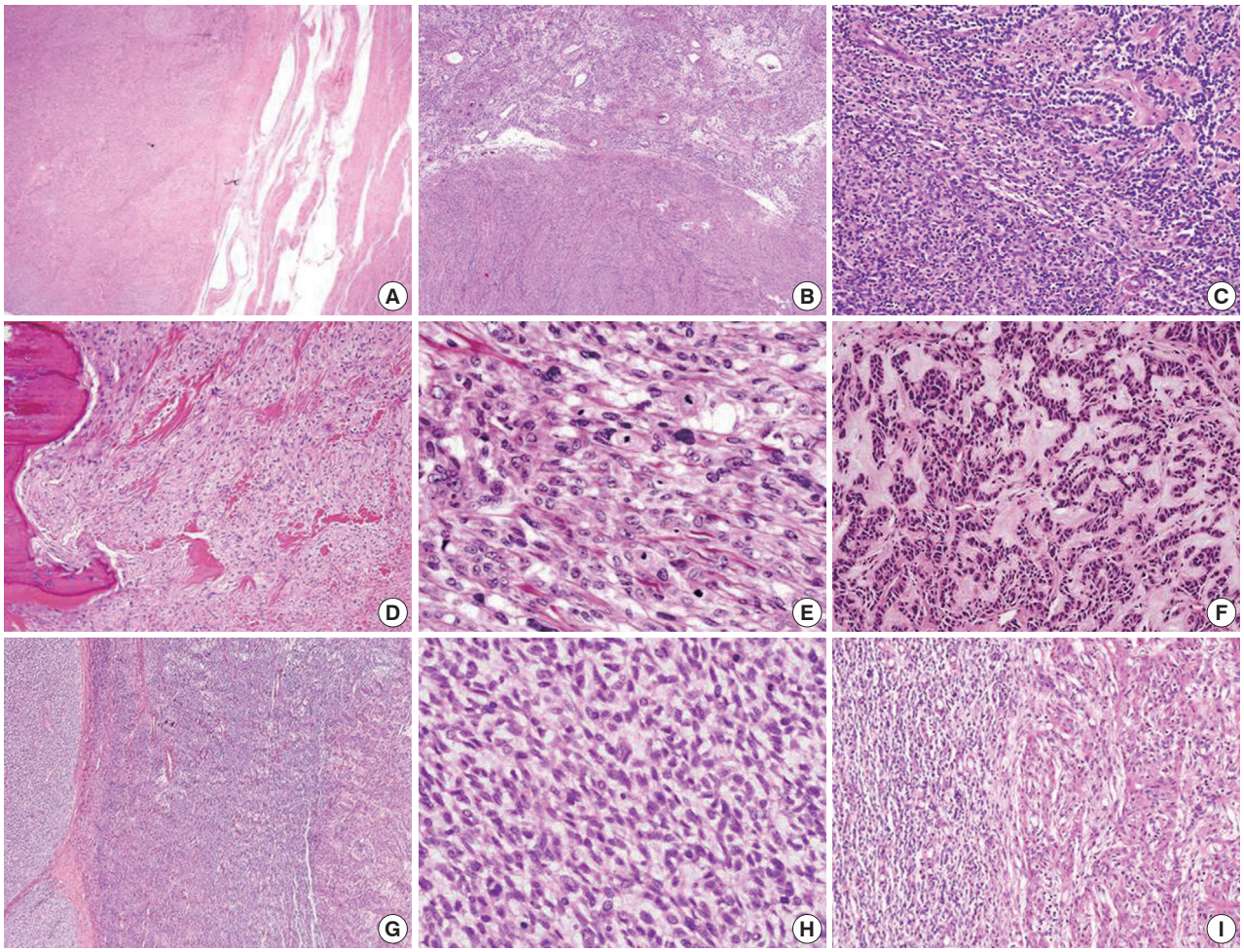


**Fig. 1.** Various types of histological differentiation in low-grade endometrial stromal sarcoma (LGESS) and undifferentiated endometrial sarcoma (UES). (A, B) LGESS with smooth muscle differentiation. Smooth muscle differentiation at the periphery of the tumor forms a well-circumscribed margin, resulting in a diagnostic difficulty from endometrial stromal nodule with smooth muscle differentiation or cellular leiomyoma (A). In another case, a discrete nodule resembling leiomyoma is observed within the classic LGESS area, creating a distinct border between the two areas (B). (C) In a tumor with sex cord-like differentiation, anastomosing trabeculae of epithelioid cells resemble an ovarian granulosa cell tumor. (D) In a tumor with osseous differentiation, osteoid material and woven bone are dispersed within the tumor obscuring the distinct vascular pattern. (E) UES shows diffuse fascicular arrangement of poorly differentiated spindle cells containing nuclear pleomorphism and frequent mitotic activity. (F) UES with sex cord-like differentiation contains trabecular arrangement of tumor cells in a chondromyxoid background. (G-I) Combined LGESS and UES. The tumor has three different portions comprised of UES, classic LGESS, and LGESS smooth muscle differentiation (G). The inner portion of the mass is composed of undifferentiated spindle cells with high cellularity and frequent mitotic counts in the myxoid stroma, but lacks a characteristic vascular pattern (H). Classic features of LGESS and smooth muscle differentiation are observed in the middle and outer portions of the tumor (I).

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 trabeculae 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.

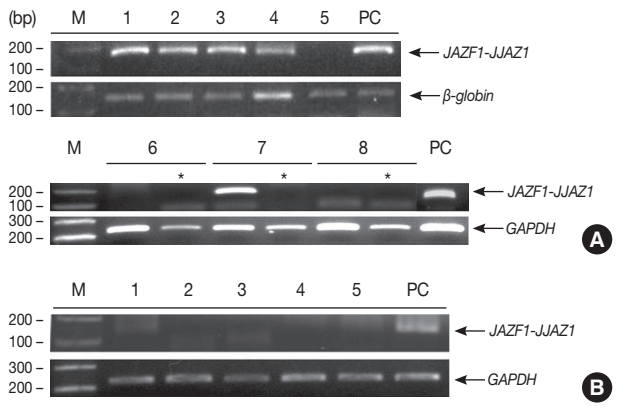


**Fig. 2.** Agarose gel electrophoresis of nested reverse transcription polymerase chain reaction in low-grade endometrial stromal sarcoma (LGESS), endometriosis, and normal endometrium cases. (A) The *JAZF1/JJAZ1* fusion transcript is detected in 18 (85.7%) of 21 LGESS cases. In LGESS with smooth muscle differentiation, the fusion transcript is detected only within the classical areas. *Differentiated area in LGESS showing smooth muscle differentiation. (B) Endometriosis (cases 1 and 2) and normal endometrium (cases 3-5) are negative for the *JAZF1/JJAZ1* fusion gene. *β-globin* or *GAPDH* serves as the control for the presence of intact RNA.

### 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.[23] 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

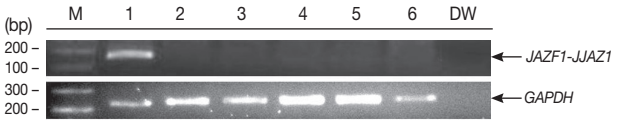


**Fig. 3.** Agarose gel electrophoresis of nested reverse transcription polymerase chain reaction for undifferentiated endometrial sarcomas (UES). The *JAZF1/JJAZ1* fusion transcript is detected in two (28.6%) of seven UES cases. *GAPDH* served as control for the presence of intact RNA. M, marker; DW, distilled water.

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

| Diagnosis | No. of cases | No. of *JAZF1/JJAZ1* fusion gene positive cases (%) |
|---|---|---|
| LGESS, classic | 15 | 14 (93.3) |
| LGESS with smooth muscle differentiation[a] | 3 | 1 (33.3) |
| LGESS with sex cord-like differentiation | 2 | 2 (100) |
| LGESS with osseous differentiation | 1 | 1 (100) |
| UES, not otherwise specified | 5 | 2 (40) |
| UES with sex cord-like differentiation | 1 | 0 (0) |
| Combined LGESS and UES | 1 | 0 (0) |

[a]Fusion gene is identified only in the classical area of LGESS.
LGESS, low-grade ESS; UES, undifferentiated endometrial sarcoma.


**Fig. 4.** Sequencing results for the *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 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;[4] 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 *JAZF1/JJAZ1* fusion gene,[20,24] but only two cases with secondary histological differentiation showed *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 should be based on histological and immunohistochemical findings, and an examination for the *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 β-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 β-catenin expression in typical cases of endometriosis and LGESS. We experienced two such cases in which endometrial-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 *JAZF1/JJAZ1* gene fusion in conjunction with other histopathological and immunohistochemical features led us to 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 *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.

The frequency of *JAZF1/JJAZ1* fusion among LGESS cases varies among several studies, ranging from 23% to 80%.[20,23-27] Our study recorded the highest prevalence rate of gene fusion among ESS cases. These variations in frequency may be explained by differences in the sensitivity of assays or RNA quality in tumor samples. False-negative results may also be ascribed to molecular heterogeneity of fusion transcripts or tumors carrying other chromosomal aberrations. False-positive results are attributed to cDNA contamination during the PCR procedure. Fluorescence *in situ* hybridization may be useful as a complementary technique to exclude the possibility of false-positive contamination and to detect molecular variations at t(7;17). In our analysis, LGESS cases with classic histology showed a high prevalence (14 of 15 cases) of *JAZF1/JJAZ1* gene fusion. Huang *et al.*[27] reported the presence of this fusion transcript in 75% of classical LGESS cases but not cases with secondary histological differentiation. However, in the current study, four of the six

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.*[23] *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.[30]

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


1. Chew I, Oliva E. Endometrial stromal sarcomas: a review of potential prognostic factors. Adv Anat Pathol 2010; 17: 113-21.
2. Crum CP, Lee KR. Diagnostic gynecologic and obstetric pathology. Philadelphia: Saunders, 2006.
3. Yilmaz A, Rush DS, Soslow RA. Endometrial stromal sarcomas with unusual histologic features: a report of 24 primary and metastatic tumors emphasizing fibroblastic and smooth muscle differentiation. Am J Surg Pathol 2002; 26: 1142-50.
4. Oliva E, Clement PB, Young RH. Endometrial stromal tumors: an update on a group of tumors with a protean phenotype. Adv Anat Pathol 2000; 7: 257-81.
5. Oliva E, Clement PB, Young RH, Scully RE. Mixed endometrial stromal and smooth muscle tumors of the uterus: a clinicopathologic study of 15 cases. Am J Surg Pathol 1998; 22: 997-1005.
6. Roth LM, Senteny GE. Stromomyoma of the uterus. Ultrastruct Pathol 1985; 9: 137-43.
7. McCluggage WG, Ganesan R, Herrington CS. Endometrial stromal sarcomas with extensive endometrioid glandular differentiation: report of a series with emphasis on the potential for misdiagnosis and discussion of the differential diagnosis. Histopathology 2009; 54: 365-73.
8. Njim L, Moussa A, Denguezli W, Hadhri R, Zakhama A. Low-grade endometrial stromal sarcoma with extensive glandular differentiation. APMIS 2008; 116: 834-6.
9. Kibar Y, Aydin A, Deniz H, Balat O, Cebesoy B, Al-Nafussi A. A rare case of low-grade endometrial stromal sarcoma with myxoid differentiation and atypical bizarre cells. Eur J Gynaecol Oncol 2008; 29: 397-8.
10. Shah R, McCluggage WG. Symplastic atypia in neoplastic and non-neoplastic endometrial stroma: report of 3 cases with a review of atypical symplastic cells within the female genital tract. Int J Gynecol Pathol 2009; 28: 334-7.
11. Kildal W, Pradhan M, Abeler VM, Kristensen GB, Danielsen HE. Beta-catenin expression in uterine sarcomas and its relation to clinicopathological parameters. Eur J Cancer 2009; 45: 2412-7.
12. Tanimoto A, Sasaguri T, Arima N, Hashimoto H, Hamada T, Sasaguri Y. Endometrial stromal sarcoma of the uterus with rhabdoid features. Pathol Int 1996; 46: 231-7.
13. Rosty C, Genestie C, Blondon J, Le Charpentier Y. Endometrial stromal tumor associated with rhabdoid phenotype and and zones of "sex cord-like" differentiation. Ann Pathol 1998; 18: 133-6.
14. Lifschitz-Mercer B, Czernobilsky B, Dgani R, Dallenbach-Hellweg G, Moll R, Franke WW. Immunocytochemical study of an endometrial diffuse clear cell stromal sarcoma and other endometrial stromal sarcomas. Cancer 1987; 59: 1494-9.
15. Lloreta J, Prat J. Endometrial stromal nodule with smooth and skeletal muscle components simulating stromal sarcoma. Int J Gynecol Pathol 1992; 11: 293-8.
16. Doghri R, Mrad K, Driss M, *et al.* Endometrial stromal sarcoma presenting as a cystic abdominal mass. Pathologica 2009; 101: 93-6.
17. Pérez-Montiel D, Salmeron AA, Domínguez Malagon H. Multicystic endometrial stromal sarcoma. Ann Diagn Pathol 2004; 8: 213-8.
18. McCluggage WG, Sumathi VP, Maxwell P. CD10 is a sensitive and

diagnostically useful immunohistochemical marker of normal endometrial stroma and of endometrial stromal neoplasms. Histopathology 2001; 39: 273-8.
19. Jung CK, Jung JH, Lee A, *et al.* Diagnostic use of nuclear beta-catenin expression for the assessment of endometrial stromal tumors. Mod Pathol 2008; 21: 756-63.
20. Kurihara S, Oda Y, Ohishi Y, *et al.* Endometrial stromal sarcomas and related high-grade sarcomas: immunohistochemical and molecular genetic study of 31 cases. Am J Surg Pathol 2008; 32: 1228-38.
21. Liao X, Wang Y, Yue C, *et al.* Highly cellular leiomyoma of uterus: a comparative morphologic and immunohistochemical study of endometrial stromal tumors. Zhonghua Bing Li Xue Za Zhi 2002; 31: 396-400.
22. Chu PG, Arber DA, Weiss LM, Chang KL. Utility of CD10 in distinguishing between endometrial stromal sarcoma and uterine smooth muscle tumors: an immunohistochemical comparison of 34 cases. Mod Pathol 2001; 14: 465-71.
23. Koontz JI, Soreng AL, Nucci M, *et al.* Frequent fusion of the JAZF1 and JJAZ1 genes in endometrial stromal tumors. Proc Natl Acad Sci U S A 2001; 98: 6348-53.
24. Hrzenjak A, Moinfar F, Tavassoli FA, *et al.* JAZF1/JJAZ1 gene fusion in endometrial stromal sarcomas: molecular analysis by reverse transcriptase-polymerase chain reaction optimized for paraffin-embedded tissue. J Mol Diagn 2005; 7: 388-95.
25. Nucci MR, Harburger D, Koontz J, Dal Cin P, Sklar J. Molecular analysis of the JAZF1-JJAZ1 gene fusion by RT-PCR and fluorescence in situ hybridization in endometrial stromal neoplasms. Am J Surg Pathol 2007; 31: 65-70.
26. Micci F, Walter CU, Teixeira MR, *et al.* Cytogenetic and molecular genetic analyses of endometrial stromal sarcoma: nonrandom involvement of chromosome arms 6p and 7p and confirmation of JAZF1/JJAZ1 gene fusion in t(7;17). Cancer Genet Cytogenet 2003; 144: 119-24.
27. Huang HY, Ladanyi M, Soslow RA. Molecular detection of JAZF1-JJAZ1 gene fusion in endometrial stromal neoplasms with classic and variant histology: evidence for genetic heterogeneity. Am J Surg Pathol 2004; 28: 224-32.
28. Tavassoli FA, Devilee P. World Health Organization classification of tumours: pathology and genetics of tumours of the breast and female genital organs. Lyon: IARC Press, 2003.
29. Kurihara S, Oda Y, Ohishi Y, *et al.* Coincident expression of beta-catenin and cyclin D1 in endometrial stromal tumors and related high-grade sarcomas. Mod Pathol 2010; 23: 225-34.
30. Oliva E, de Leval L, Soslow RA, Herens C. High frequency of JAZF1-JJAZ1 gene fusion in endometrial stromal tumors with smooth muscle differentiation by interphase FISH detection. Am J Surg Pathol 2007; 31: 1277-84.