

Extrauterine endometrial stromal sarcoma with *JAZF1/JJAZ1* fusion confirmed by RT-PCR and interphase FISH presenting as an inguinal tumor

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Abstract Endometrial stromal sarcomas are rare malignant mesenchymal tumors that usually develop in the uterine corpus and occasionally arise at various extrauterine sites. This report describes the first case of primary extrauterine endometrial stromal sarcoma arising in the extraperitoneal portion of the round ligament presenting as a solitary inguinal mass in a 46-year-old woman. The patient presented gradually growing tumor in the right inguinal region. Local tumor resection was performed and no recurrence or metastasis was found at 15 months after the operation. Histological examination revealed that the tumor comprised uniform, spindle-shaped cells with blunt nuclear figure and scattered small arteries, and infiltrated into adjacent tissue. No endometriosis was morphologically identified in the lesion. Immunohistochemically, the tumor cells were positive for CD10, estrogen receptor, progesterone receptor, α -smooth muscle actin, and calponin. We

confirmed *JAZF1/JJAZ1* fusion by reverse transcription–polymerase chain reaction and the corresponding chromosomal translocation by interphase fluorescence in situ hybridization on paraffin sections. It is essential that the inguinal region should be recognized as a possible primary site of endometrial stromal sarcoma, and the detection of a *JAZF1/JJAZ1* fusion can be useful when the diagnosis is not confirmed by microscopic observation or immunohistochemistry for the tumor arising in extrauterine sites.

Keywords Endometrial stromal sarcoma · Inguinal hernia · Round ligament · *JAZF1/JJAZ1* · Interphase FISH

Introduction

Endometrial stromal sarcoma (ESS) is a rare type of uterine tumor that accounts for less than 10% of uterine sarcomas [10]. The World Health Organization's classification of tumors of the breast and female genital organs subclassified endometrial stromal tumors into three categories: endometrial stromal nodule, low-grade ESS, and undifferentiated endometrial sarcoma (UES) [3]. The tumors traditionally called high-grade ESS were recently reclassified as UES due to their lack of specific differentiation and histological resemblance to endometrial stroma [3]. Accordingly, ESS only means low-grade ESS. ESS was distinguished from the endometrial stromal nodule based on the presence of an infiltrative growing character or vascular invasion in ESS [4, 5, 7, 8].

Recently, a fusion of two zinc finger genes, *JAZF1* and *JJAZ1*, caused by a reciprocal translocation $t(7;17)(p15;q21)$ was detected in more than 80% of ESS cases [4, 5, 7, 8]. Furthermore, *JAZF1/PHF1* or *EPC1/PHF1* fusion also presented in a small number of ESS cases [9]. Diagnosis of

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ESS has been dependent on histological findings supported by immunohistochemical examination. However, it could be difficult to get to a definite diagnosis for monophasic spindle cell tumors, especially when an unusual location was involved. Molecular detection of specific fusion transcripts could overcome these problems and should become a potent diagnostic tool for ESS.

ESS's usually develop as primary uterine tumor, but ESS's of extrauterine origin, such as the ovary, fallopian tube, pelvic cavity, abdominal cavity, omentum, retroperitoneum, vagina, vulva, and sigmoid colon, have been reported [2, 6]. The round ligament passes through the inguinal canal and comprises mainly smooth muscle, fibrous tissue, blood vessels, and nerves [12]. Most round ligament tumors are leiomyomas or fibromas, and there is no previous report of ESS arising in the inguinal portion of the round ligament.

We described the first case of primary extrauterine ESS arising from the round ligament in the inguinal canal, unassociated with endometriosis, with a confirmed *JAZF1/JJAZ1* fusion by reverse transcription–polymerase chain reaction (RT-PCR) and interphase fluorescence in situ hybridization (FISH) analysis.

Clinical history

A 46-year-old woman, para 3 and gravida 3, presented with an indolent mass in the right inguinal region. Her menstrual cycle was regular, and her gynecologic history was unremarkable. Physical examination suggested an inguinal hernia. The MRI showed a demarcated 3.0-cm solid mass in the right inguinal canal (Fig. 1a), and no significant lesion in the uterus. Tumor resection was performed. At the operation, the tumor was not covered with a hernia sac nor adhered to the peritoneum but attached to the extraperitoneal portion of the round ligament. There was no evidence of recurrence or metastasis 15 months after surgery.

Materials and methods

Surgically resected specimen was fixed in 10% neutral-buffered formalin, cut into 6 tissue blocks of 5 mm in thickness, and embedded in paraffin. Sections, 4- μ m thick, were used for hematoxylin and eosin staining, immunohistochemical studies, molecular analysis, and interphase FISH.

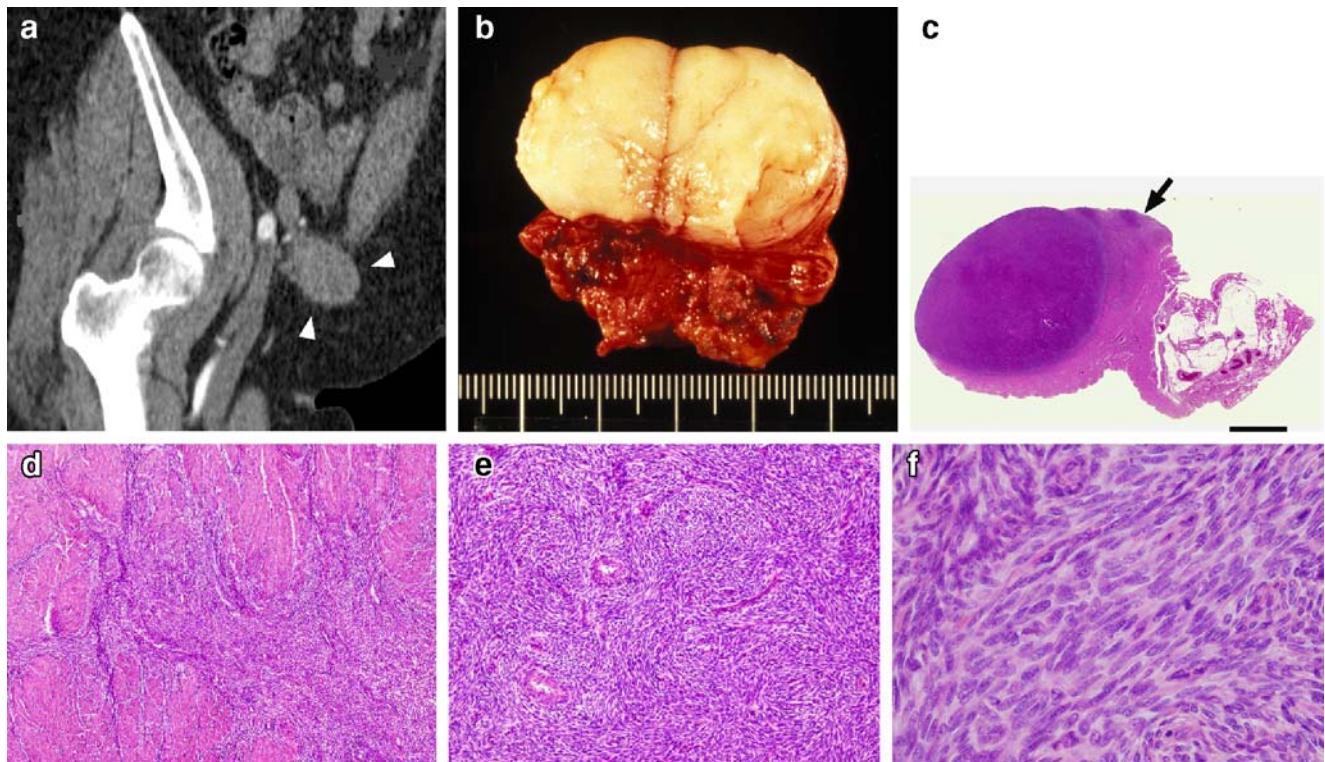


Fig. 1 **a** MRI showing a mass (*arrow head*) in the right inguinal canal. **b** Macroscopic appearance of the cut surface of the tumor showing a yellow-white tumor. **c** Panoramic view of the tumor demonstrating invasion to adjacent tissue (*arrow*). H & E staining; scale bar, 5 mm. **d** The tumor cells infiltratively growth into the

adjacent smooth muscle tissue of the round ligament. H & E staining (original magnification $\times 40$). **e** The tumor is densely cellular, and whorls around small arteries are observed. H & E staining (original magnification $\times 100$). **f** The tumor cells are uniform, short, and spindle shaped. H & E staining (original magnification $\times 400$)

Immunohistochemistry

Immunohistochemical examinations were performed on deparaffinized thin sections using the standard avidin–biotin–peroxidase complex method with an automated immunostainer (Ventana Medical System, Tucson, AZ, USA). Primary antibodies against following antigens were used: low molecular weight cytokeratin (CAM5.2, prediluted, Becton–Dickinson, San Jose, CA, USA), high molecular weight cytokeratin (34 β E12, 1:75, Dako, Glostrup, Denmark), vimentin (V9, 1:100, Dako), α -smooth muscle actin (1A4, 1:200, Dako), desmin (D33, 1:100, Dako), calponin (CALP, 1:75, Dako), high-molecular-weight caldesmon (h-caldesmon; h-CD, 1:100, Dako), bcl-2 (124, 1:50, Dako), E-cadherin (NCH-38, 1:100, Dako), epithelial membrane antigen (EMA; E29, 1:100, Dako), CD10 (56C6, 1:20, Novocastra, Newcastle upon Tyne, UK), CD34 (NU-4A1, 1:100, Nichirei, Tokyo, Japan), CD117 (c-kit; polyclonal, 1:80, Dako), estrogen receptor (1D5, 1:50, Dako), progesterone receptor (PgR636, 1:800, Dako), calretinin (polyclonal, prediluted, Invitrogen, Carlsbad, CA, USA), inhibin- α (R1, 1:50, Dako), S-100 protein (2A10, Immuno-Biological Laboratories, Gunma, Japan), and Ki-67 (MIB-1, 1:50, Dako). Antigen retrieval was carried out with appropriate methods for each antibody.

Molecular analysis

Three sections cut from paraffin-embedded tissue were prepared for RT-PCR. The RNA extraction and RT-PCR to identify the *JAZF1/JJAZ1* fusion was carried out as previously described using the following primers: JAZF1-369-FW (5'-CCACCCATCACCCCCTCCT-3') and JJAZ1-400-RV (5'-TGCTATGAGATTCGAGTTC-3') [5]. To confirm the *JAZF1/JJAZ1* fusion, PCR products extracted from a corresponding band in agarose gel were analyzed by direct sequencing. The RT-PCR for detection of *SYT/SSX* fusion transcripts, frequently observed in synovial sarcoma, were performed as reported elsewhere [11, 13].

Interphase fluorescence in situ hybridization

The interphase FISH was performed on the paraffin-embedded tissue sections. The probes used were derived from bacterial artificial chromosomes (BAC). BAC clones were commercially purchased from Invitrogen. To detect chromosomal translocations, *JAZF1/JJAZ1* fusion, the probes were selected as follows: RP11-350G16 encompassing the entire *JAZF1* gene mapping to 7p15 and CTD-3205H10 fully covering the *JJAZ1* gene mapping to 17q21 according to the mapping data at the NCBI map viewer website (<http://www.ncbi.nlm.nih.gov/mapview/static/MVstart.html>). RP11-350G16 and CTD-3205H10 were

labeled with digoxigenin and biotin, respectively, with Nick translation mix (Roche Diagnostics, Basel, Switzerland). The slides were deparaffinized in xylene and rehydrated in ethanol. The slides were pretreated by microwaving in 10-mmol citrate buffer (pH 6.0) at 90°C for 15 min. The extracellular matrix in the tissue section was digested using 0.2% pepsin (in 0.01 NHCl) for 10 min at 37°C. The DNA in the tissue sections and the probes were codenatured for 10 min at 90°C, and incubated in a humidified box at 37°C for 48 h. Digoxigenin-labeled probes were detected with rhodamine-conjugated antidigoxigenin antibody, and biotinylated probes were detected with conjugated avidin (Alexa Fluor 488, Molecular Probes, Eugene, OR, USA). The nuclei were counterstained with mounting medium containing 4',6-diamidino-2-phenylindole (Vector, Burlingame, CA, USA). The probe signals were detected using a fluorescence microscope with a digital camera, with rhodamine as red and Alexa Fluor 488 as green label. For each slide, more than 100 nuclei of tumor cells were analyzed. The composed images were reconstructed with Adobe Photoshop 6.0 software (Adobe, San Jose, CA, USA).

Results

Macroscopically, the tumor was demarcated, adhered to fibromuscular tissue of the round ligament, and had a maximum diameter of 3 cm. The cutting surface of the tumor displayed a yellow-white color and had an elastic hard consistency (Fig. 1b). No necrosis or hemorrhage was apparent.

Microscopically, the tumor cells infiltrated the adjacent smooth muscle tissue of the round ligament at the periphery, 10 mm away from the main mass (Fig. 1c and d). The tumor was highly cellular, and whorls of the tumor cells around small arteries were observed (Fig. 1e). The tumor cells were uniform and spindle-shaped, and had bland nuclei and indistinct cytoplasmic margins (Fig. 1f). Mitotic figures were rare. There was no evidence of vascular invasion or endometriosis throughout the entire specimen.

Immunohistochemically, the tumor cells were positive for CD10, estrogen receptor, progesterone receptor, bcl-2, vimentin, α -smooth muscle actin, and calponin but negative for low and high molecular weight cytokeratin, desmin, h-caldesmon, E-cadherin, EMA, CD34, CD117, calretinin, inhibin- α , and S-100 protein. Less than 1% of tumor cells were positive for Ki-67.

The RT-PCR for the detection of the *JAZF1/JJAZ1* fusion transcript showed a fusion product at the expected level of 93 bp as previously reported (Fig. 2a). The fusion of *JAZF1* and *JJAZ1* genes was confirmed by direct sequencing (Fig. 2b). No *SYT-SSX1* or *SYT-SSX2* fusions were detected.

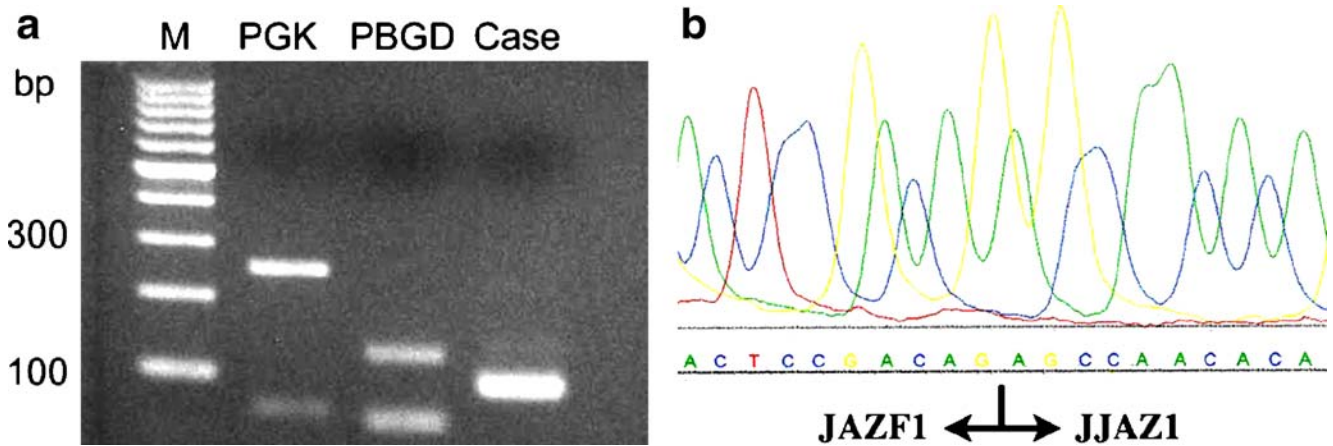


Fig. 2 Detection of the *JAZF1/JJAZ1* fusion transcript. **a** Fusion transcript is amplified and identified in the expected range of 93 bp. **b** Partial chromatogram showing the junction of the *JAZF1/JJAZ1* fusion

gene (arrows). *M* 100-bp DNA ladder, *PGK* phosphoglycerate kinase (247 bp, internal control), *PBGD* porphobilinogen deaminase (127 bp, internal control), *Case* RNA extracted from the present tumor

The interphase FISH revealed a *JAZF1/JJAZ1* fusion as a result of a reciprocal translocation between 7p15 and 17q21 (Fig. 3).

Discussion

ESS's usually arise in the uterine corpus and are composed of uniform ovoid to slightly spindle-shaped cells. However, these tumors occasionally present elongated spindle-shaped cells that are hard to distinguish from various spindle cell tumors and may be confused with other tumors. In this case, histological diagnosis was difficult because the tumor developed in the extrauterine site, the inguinal region, and comprised uniform, elongated spindle-shaped cells.

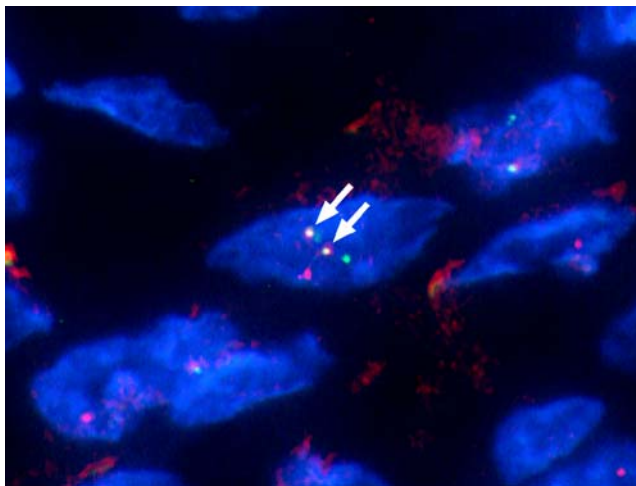


Fig. 3 Interphase FISH analysis on a paraffin section. Two fusion spots (merge signal, arrows), one red spot and one green spot, indicate *JAZF1/JJAZ1* fusion originating from a reciprocal translocation between 7p15 and 17q21

Immunohistochemical findings supported the differential diagnosis for spindle cell tumor. The present tumor was positive for CD10, estrogen receptor, progesterone receptor, α -smooth muscle actin, calponin but negative for desmin and h-caldesmon. These findings supported the diagnosis of ESS. Monophasic fibrous synovial sarcoma (MFSS) is a representative spindle-shaped soft tissue tumor that resembles ESS histologically, demonstrating *SYT/SSX1* or *SYT/SSX2* chimera gene in more than 90% of the cases [13]. In this case, although these chimera genes were not revealed by RT-PCR using RNA extracted from paraffin-embedded tissues, the diagnosis of MFSS could not completely be excluded from these negative results only.

Previous studies examining the genetic aspects of ESS's revealed frequent chromosomal abnormalities of 6p, 7p, and 17q [8]. In recent years, a specific chromosomal rearrangement $t(7;17)(p15;q21)$ resulting in a *JAZF1/JJAZ1* fusion transcript was reported in ESS [4, 5, 7, 8]. The *JAZF1/JJAZ1* fusion in ESS was described in 5 of 5 cases by Koontz et al. [7], 1 of 3 cases by Micci et al. [8], 3 of 13 cases including variant types by Huang et al. [4], and 16 of 20 cases by Hrzenjak et al. [5]. Micci et al. [9] identified ESS cases with two other genetic alterations, $t(6;7)(p21;p15)$ leading to a *JAZF1/PHF1* fusion gene and $t(6;10;10)(p21;q22;p11)$ causing a *EPC1/PHF1* fusion gene. The *JAZF1*, *JJAZ1*, and *PHF1* genes all encode zinc finger domains often found in DNA-binding proteins, and fusion genes previously reported in ESS, *JAZF1/JJAZ1*, *JAZF1/PHF1*, and *EPC1/PHF1* could express unusual proteins with zinc finger motifs. Wild-type *JAZF1* is expressed in normal endometrium, and loss of expression for normal *JAZF1* was demonstrated in some ESS cases [7]. The function of the *JAZF1* gene has not been elucidated, but it has been suggested that *JAZF1* might have a role in tumor suppression, and abnormal fusion proteins with DNA-

binding activities could deregulate the transcriptional process of other genes [7, 9]. The recognition of the *JAZF1/JJAZ1* fusion gene was crucial and unique in diagnosing ESS. The detection of these chimera genes should become a specific diagnostic tool, especially when the ESS arises at an extrauterine site. In this case, *JAZF1/JJAZ1* fusion was confirmed by RT-PCR and interphase FISH.

Primary ESS's have been reported in various extrauterine locations [2, 6]. Irvin et al. [6] reported a case of ESS that occurred in the endometriosis of the vulva in association with the extrapelvic portion of the round ligament; however, an ESS arising from the round ligament in the inguinal canal has not been described. Some cases of extrauterine ESS included endometriosis, but many of the extrauterine ESS's described in the literature have been unassociated with endometriosis [2]. There are many reports of inguinal endometriosis often presenting as an inguinal hernia, and approximately 90% of these cases have right-side lesion [1]. The present case showed a right groin mass, but there was no endometriosis within the lesion as determined by histological examination.

In conclusion, it is important to recognize that ESS can develop in extrauterine sites such as the inguinal region, and the detection of *JAZF1/JJAZ1* fusion can be useful when the diagnosis is not definitive by other studies including light microscopy and immunohistochemistry.

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