Human Pathology (2021) **109**, 31–36

ELSEVIER

Human PATHOLOGY

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Original contribution

Detection of *MED12* mutations in mesenchymal components of uterine adenomyomas

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Received 21 October 2020; revised 20 November 2020; accepted 23 November 2020

Available online 28 November 2020

Keywords:
Adenomyoma;
Uterus;
MED12;
Mutation;
Microdissection

Summary Adenomyoma of the uterus is a biphasic nodular lesion composed of a mesenchymal component with smooth muscle differentiation and a glandular epithelium. The neoplastic nature of uterine adenomyomas has been controversial because some are considered to be nodular adenomyosis. *MED12* mutations are involved in the pathogenesis of uterine smooth muscle tumors (leiomyomas and leiomyosarcomas) and biphasic tumors of the breast (fibroadenomas and phyllodes tumor). To investigate the histogenesis of uterine adenomyomas, we performed pathological and genetic analyses, including Sanger sequencing of *MED12*. In total, 15 cases of uterine adenomyomas were retrieved and assessed for clinicopathological factors. Immunohistochemistry for smooth muscle actin, desmin, and CD10 was performed. Exon 2 of *MED12* was Sanger sequenced using DNA obtained by macrodissection of the adenomyomas. For cases that were positive for somatic *MED12* mutations, we next performed microdissection of the mesenchymal and epithelial components. The DNA extracted from each component was further analyzed for *MED12* mutations. *MED12* mutations were detected in two adenomyomas (2/15, 13%), all in a known hot spot (codon 44). In both lesions, *MED12* mutations

Competing interest: The research of D.M. is partly supported by Takara Bio Inc.
Funding support: This work was supported by the following grants: JSPS KAKENHI [Grant Number JP 26870129 (D.M.) and 25460431 (A.G.)]
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https://doi.org/10.1016/j.humpath.2020.11.013

were detected in multiple spots of the mesenchymal component. The epithelial component did not harbor *MED12* mutations. The relatively low frequency of *MED12* mutations suggests that not all adenomyomas are leiomyomas with entrapped glands. However, the results of our study suggest that a subset of uterine adenomyomas are true mesenchymal neoplasms.

1. Introduction

Uterine adenomyomas are tumor-like masses composed of endometrial glands, stroma, and smooth muscle that might be located within the myometrium, or they may involve or originate in the endometrium and grow as a polyp [1,2]. The current classification of the World Health Organization categorizes adenomyoma in the mixed epithelial and mesenchymal tumors category, together with atypical polypoid adenomyoma and adenosarcoma [3]. However, there has been controversy over whether adenomyoma is a non-neoplastic lesion (nodular adenomyosis) or a biphasic neoplasm characterized by the proliferation of smooth muscle and a glandular epithelium [1]. In our experience, some adenomyomas have minor glandular components only at the periphery. Such lesions may be considered leiomyomas with entrapped glands.

Recent molecular analyses have made considerable contributions to our understanding of the pathogenesis of uterine biphasic lesions. For example, aberrant expression of cancer-associated genes such as *PTEN* was documented in glandular components of an atypical polypoid adenomyoma, suggesting their glandular components to be precancerous [4]. There has also been a microdissection-based analysis which proved *CTNNB1* mutation in their glandular components [5]. Adenosarcomas, by contrast, are now regarded as pure mesenchymal neoplasms based on the results of a microdissection-based sequence analysis, which revealed the presence of somatic oncogenic mutations exclusively in the mesenchymal component. Their mesenchymal component is considered to be malignant, whereas the epithelial component is benign and non-neoplastic [6]. Regarding uterine adenomyomas, molecular evidence is sparse and prior mutational analyses did not focus on the distinction between the epithelial and mesenchymal components [7–9].

MED12 is a component of the Mediator complex, which is the major regulator of the transcription of a plethora of genes [10]. Its somatic mutation is detected in approximately 70% and 10% of uterine leiomyomas and leiomyosarcomas, respectively [11]. Extrauterine smooth muscle tumors in women such as those arising in the retroperitoneum and ovary are also known to harbor *MED12* mutation [12,13]. Of interest, *MED12* mutations are also frequently identified in breast fibroepithelial lesions such as fibroadenomas and phyllodes tumor [14,15]. The somatic alterations in these tumors are restricted to the mesenchymal component and absent in the epithelium [16,17].

Considering that smooth muscle proliferation and a mixed mesenchymal and epithelial morphology are hallmark features of uterine adenomyomas, we hypothesized that *MED12* mutations are involved in their pathogenesis. To test this hypothesis, microdissection-based molecular analyses of uterine adenomyomas together with clinicopathological examinations were conducted.

2. Materials and methods

2.1. Case selection and pathological evaluation

We retrieved and reviewed hematoxylin and eosin-stained sections of 15 cases of adenomyomas that were surgically removed. The diagnosis of uterine adenomyomas was made by two of the authors (M.K. and D.M.). Adenomyomas were required to exhibit the following features: (1) nodularity of the tumor demonstrated radiographically, macroscopically, or microscopically and (2) benign endometrial glands with or without a stroma surrounded by leiomyomatous smooth muscle. For hysterectomy specimens, the presence or absence of background adenomyosis was evaluated. Clinical information related to these lesions was gathered from charts. Ethical approval was obtained from the relevant institutional review boards (approval nos. 1211 and 858 for Akita University and Osaka University, respectively).

2.2. Immunohistochemistry

All tissue samples were fixed in formalin and embedded in paraffin. Full tissue sections (4 μm thick) were used for immunohistochemistry in all cases. Immunohistochemical staining was performed according to standard techniques on a Ventana Benchmark XT Autostainer (Ventana Medical Systems Inc., Tucson, AZ). We applied three antibodies: CD10 (1:50, anti-mouse, Clone 56C6; Dako, Glostrup, Denmark), desmin (1:150, anti-mouse, Clone DE-R-11; Leica, Newcastle, UK), and smooth muscle actin (SMA) (1:4000, anti-mouse, Clone 1A4; Dako). Appropriate positive and negative controls were included.

2.3. DNA extraction and sequencing of *MED12*

We analyzed all 15 cases of uterine adenomyomas for *MED12* mutations. Sections (10 μm thick) were cut from paraffin-embedded blocks containing adenomyomas. Representative areas of the adenomyomas were macrodissected. Extraction of genomic DNA was performed using a PicoPure DNA Extraction Kit (Life Technologies Corp., Carlsbad, CA). Because most previously reported *MED12* mutations were clustered in exon 2 and intron 1, we analyzed this region by polymerase chain reaction (PCR) followed by direct sequencing. PCR was performed using two sets of primer pairs: (1) F: 5′-GCCCTTTCACCTTGTTCCTT-3′ and R: 5′-AAGCTGACGTTCTTGGCACT-3′ and (2) F: 5′-AACAACTAAACGCCGCTTTC-3′ and R: 5′-AAGCTGACGTTCTTGGCACT-3'. For cases positive for *MED12* mutations, we next performed microdissection of the mesenchymal and epithelial components of the adenomyomas in the toluidine blue-stained 10-μm-thick sections. The DNA extracted from each component was analyzed for *MED12* mutations as described previously. Amplification was performed using a T100TM Thermal Cycler (Bio-Rad, Hercules, CA). An Applied Biosystems 3730xl DNA Analyzer (Foster City, CA) was used for sequence analysis.

3. Results

3.1. Clinicopathological features and pathological findings of the uterine adenomyomas

Of the 15 cases, based on the clinical, radiographic, and macroscopic findings, we designated eight adenomyomas as intramural, six as polypoid/submucosal, and one as a subserosal mass (Table 1). Six adenomyomas (40.0%) were identified in the hysterectomy specimens. The remaining adenomyomas were enucleated and removed by polypectomy or resectoscopic myomectomy.

Microscopically, in all of the cases, endometrial-type glands were located within the stroma composed of smooth muscle cells. In a few cases, the glands were located predominantly at the periphery of the nodule. The amount of endometrial stroma surrounding the glands varied between the cases. The glandular epithelium within the adenomyomas lacked atypia. In four of six hysterectomy cases, adenomyosis was observed in the background myometrium.

Positive immunoreactivity for SMA and desmin was observed in the mesenchymal component of the adenomyomas in all cases. CD10-positive stromal cells were observed around the glandular epithelium.

3.2. Mutational analysis of *MED12* in adenomyomas

Sanger sequencing of *MED12* using the DNA extracted from macrodissected tissues of uterine adenomyomas revealed mutations in a known hot spot (codon 44, nucleotide 131) in two cases (2/15, 13.3%). These *MED12*-mutated adenomyomas were intramural and submucosal.

The intramural adenomyoma with a *MED12* mutation (case 3) was a well-circumscribed and enucleated nodular lesion (Fig. 1). We selectively microdissected glandular epithelium within the glands, in the areas where they showed telescoping, to avoid contamination of smooth muscle component. The mesenchymal component (smooth muscle component) was microdissected separately from three distant areas (two representative areas are shown in Fig. 1). Sanger sequencing using the DNA extracted from the microdissected tissues revealed the presence of a *MED12* mutation (c.131G > A) in mesenchymal components from all three areas, but no mutation was detected in the glandular component.

Table 1 Clinicopathological data and results of the MED12 mutation analysis.

Case	Age	Location	Type of surgery	Background adenomyosis	MED12 status
1	68	Intramural	Hysterectomy	+	WT
2	66	Intramural	Hysterectomy	+	WT
3	31	Intramural	Enucleation	NE	Mutant (c.131G > A)
4	37	Intramural	Enucleation	NE	WT
5	29	Intramural	Enucleation	NE	WT
6	37	Intramural	Enucleation	NE	WT
7	32	Intramural	Enucleation	NE	WT
8	33	Intramural	Enucleation	NE	WT
9	48	Submucosal	Hysterectomy	−	Mutant (c.131G > A)
10	43	Submucosal	Enucleation	NE	WT
11	41	Submucosal	Resectoscopic myomectomy	NE	WT
12	45	Polyp	Hysterectomy	+	WT
13	45	Polyp	Hysterectomy	+	WT
14	36	Polyp	Polypectomy	NE	WT
15	70	Subserosal	Hysterectomy	+	WT

NE, not evaluable; WT, wild type.

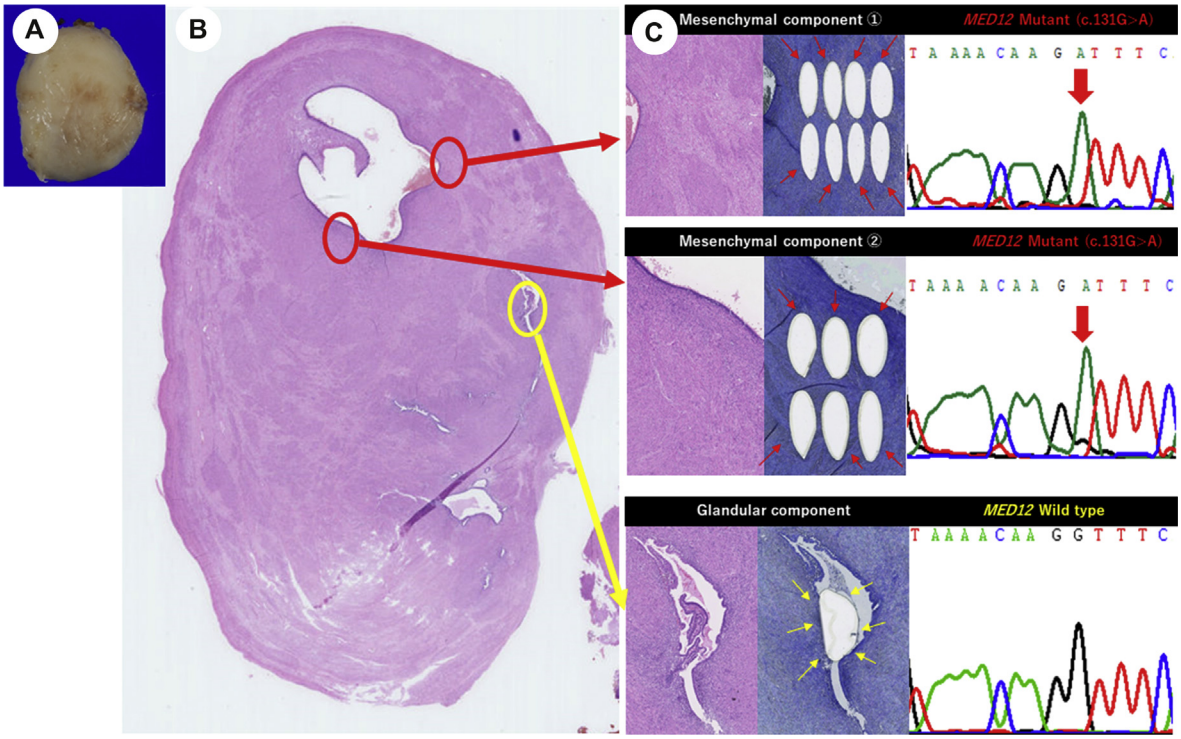

Fig. 1 (A) Macroscopically, the adenomyoma in case 3 was a nodular enucleated lesion. (B) Low-power view of the adenomyoma; scattered glands of variable sizes are observed within a nodule predominantly composed of smooth muscle. (C) High-power view of the microdissected areas of mesenchymal and glandular components and the results of *MED12* sequencing. The *MED12* mutation (c.131G > A) was detected in the mesenchymal components but not in the glandular component.

Another *MED12*-mutated adenomyoma (case 9) was a submucosal nodule identified in a hysterectomy specimen (Fig. 2). It protruded into the endometrial cavity. The lesion was composed roughly of two areas, an adenomyomatous area and a leiomyomatous area. Endometrial glands were present in the former area, whereas the latter area had no glands and resembled a leiomyoma. The mesenchymal component was microdissected from each area, as was the glandular component in the adenomyomatous area. Subsequent *MED12* sequencing revealed identical mutations (c.131G > A) in the mesenchymal components of both areas. The glandular component was wild type for *MED12*.

4. Discussion

Adenomyoma was documented initially in 1896 [1,2], and, to date, pathologists have given a diagnosis of adenomyoma for lesions that show a nodular appearance consisting of a smooth muscle component and glands with a bland morphology. Little effort has been made to elucidate their histogenesis, possibly due to their benign nature. Although the term *adenomy-oma* implies neoplasticity, whether adenomyoma is a true neoplastic lesion or not has been a topic of debate. Many researchers and pathologists have considered uterine adenomyoma as a focal adenomyosis accompanied by nodular smooth muscle proliferation and avoided making a clear distinction between adenomyoma and adenomyosis [18]. Adenomyosis, in fact, shows a variable degree of nodularity. Therefore, lesions diagnosed as adenomyomas could well be an extremely nodular variant of focal adenomyosis. However, most intramural, submucosal, and subserosal adenomyomas are nodules clearly demarcated from the surrounding myometrium and macroscopically indistinguishable from fibroids. This notion has led us to hypothesize that neoplastic smooth muscle proliferation is the essence of a subset of adenomyomas.

MED12 exon 2 mutations have been identified in a majority of uterine leiomyomas, with the reported frequencies being 48%–92% [11,19–21]. Missense and inflame insertion deletion mutations in exon 2 and adjacent intron 1 were commonly identified, with a predominance of single base substitution in codon 44. Other genomic alterations such as overexpression of *high mobility group AT-hook* (*HMGA*) and biallelic inactivation of *fumarate hydratase* (*FH*) are also known to be involved in tumorigenesis of uterine leiomyomas [23–25]. Furthermore, *MED12* mutations were identified at a high frequency in breast fibroadenoma and phyllodes tumor cases [14–17]. As common features, these are mesenchymal and sex-specific tumors. This suggests that *MED12* mutations are associated with mesenchymal, sex-specific, and organ-specific tumorigenesis. In our study, *MED12* mutations were present in 2 of 15 (13.3%) of uterine adenomyomas.

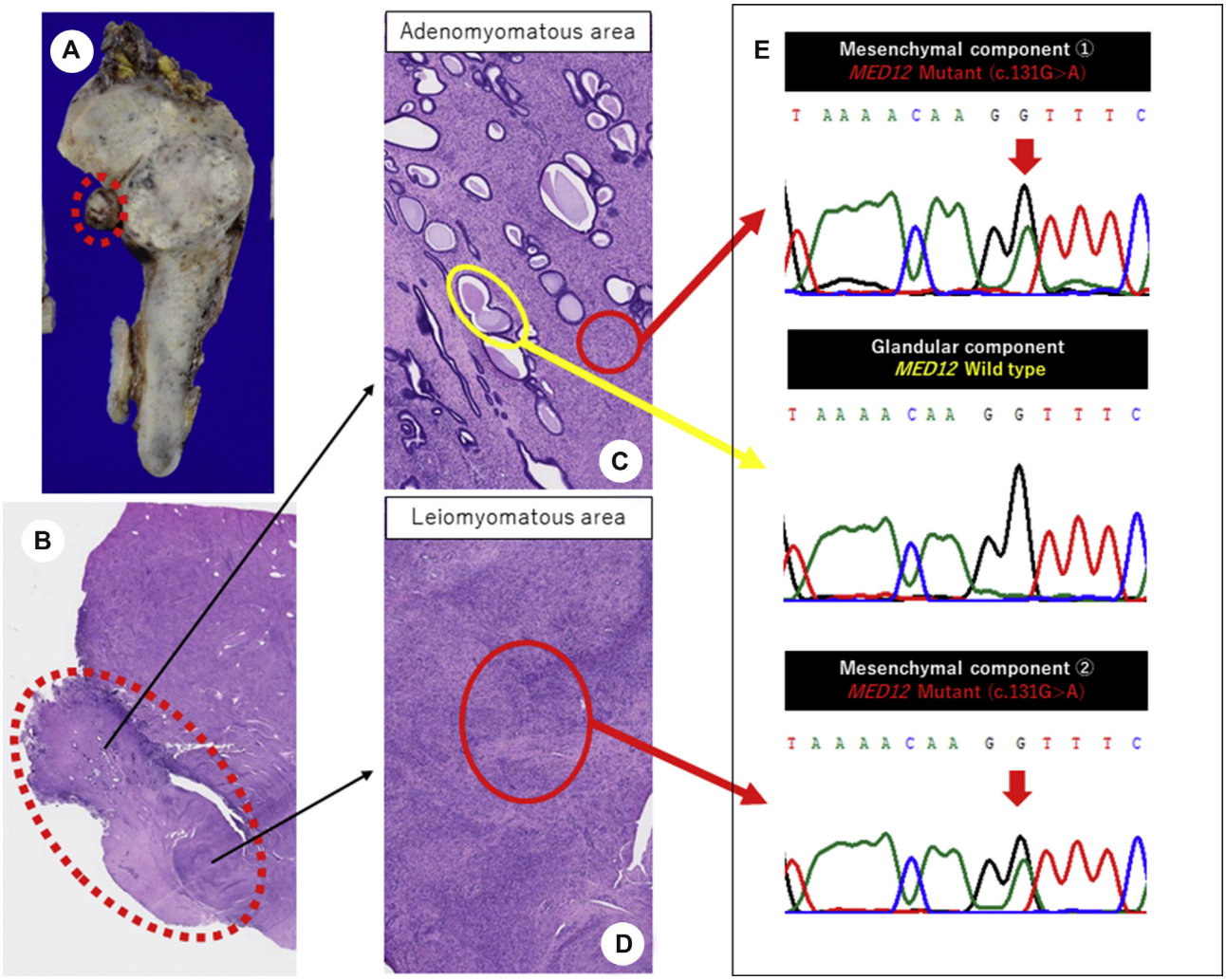

Fig. 2 (A) Macroscopically, the adenomyoma in case 9 was a submucosal nodule that protruded into the endometrial cavity. (B–D) Histologically, the adenomyoma consisted of an adenomyomatous area abundant in glands and a leiomyomatous area where glands were absent. (E) The results of *MED12* sequencing. The *MED12* mutation (c.131G > A) was detected in the mesenchymal components but not in the glandular component.

This frequency is similar to that reported in a previous study by Heikkinen et al. [8]. They assessed the status of three genes (*MED12*, *FH*, and *HMGA2*) in 21 adenomyomas and identified somatic *MED12* mutations (codon 44) in two cases and germline *FH* mutation in one, suggestive of hereditary leiomyomatosis and renal cancer syndrome. HMGA2 abnormality was not observed in any of the cases. The relatively low frequency of *MED12* mutation suggests that not all adenomyomas are leiomyomas with entrapped glands—some of them could be nodular adenomyosis. The glandular component of adenomyosis was reported to harbor *KRAS* mutations in 26 of 70 (37.1%) cases [22], and the *KRAS*-mutated clones were also detected in the normal endometrium. Future studies on *KRAS* mutations in the glandular components of adenomyomas and background adenomyosis may reveal their relationship and enhance our understanding of the histogenesis of adenomyomas.

Exophytic proliferations composed of a myomatous stroma admixed with endometrial glands have been termed variably as adenomyomatous polyps, polypoid adenomyomas, and pedunculated adenomyomas. It is of interest whether these polypoid lesions are endometrial polyps with extensive smooth muscle differentiation or whether they are the same type of lesions as the intramural adenomyomas. *MED12* mutations are extremely rare in endometrial polyps [26,27]. Although a *MED12* mutation was documented in a single case of an adenomyomatous polyp, Strickland et al. [7] recently reported the absence of a *MED12* mutation in 53 cases of adenomyomatous polyps. In our series, all three adenomyomas that presented as polyps were wild type for *MED12*. Based on this previous evidence and our results, we speculate that adenomyomatous polyps may potentially be different from adenomyomas arising from the myometrium (*i.e.*, submucosal, intramural, and subserosal

adenomyomas). However, the number of cases assessed is limited, and further investigation using a larger case series is needed to address this issue.

In conclusion, uterine adenomyoma is biphasic nodular lesion composed of a mesenchymal component with smooth muscle differentiation and a glandular epithelium of bland morphology. Mutations in *MED12*, which is frequently mutated in uterine smooth muscle tumors, were detected in 2 of 15 uterine adenomyomas. A *MED12* mutation was detected only in the mesenchymal component of uterine adenomyomas, suggesting that a subset of adenomyomas is true mesenchymal neoplasms.

Acknowledgments

A part of this work was presented at the 2017 Annual Meeting of the United States and Canadian Academy of Pathology.

References

1 Gilks CB, Clement PB, Hart WR, Young RH. Uterine adenomyomas excluding atypical polypoid adenomyomas and adenomyomas of endocervical type: a clinicopathologic study of 30 cases of an underemphasized lesion that may cause diagnostic problems with brief consideration of adenomyomas of other female genital tract sites. Int J Gynecol Pathol 2000;19:195−205. https://doi.org/10.1097/00004347-200007000-00001.

2 Tahlan A, Nanda A, Mohan H. Uterine adenomyoma: a clinicopathologic review of 26 cases and a review of the literature. Int J Gynecol Pathol 2006;25:361−5. https://doi.org/10.1097/01.pgp.0000209570.08716.b3.

3 WHO Classification of Tumours Editrial Board. In: World Health Organization classification of tumors of female reproductive organs. 5th ed. Lyon: IARC Press; 2020.

4 Němejcová K, Kenny SL, Laco J, Škapa P, Staněk L, Zikán M, et al. Atypical polypoid adenomyoma of the uterus: an immunohistochemical and molecular study of 21 cases. Am J Surg Pathol 2015;39:1148−55. https://doi.org/10.1097/PAS.0000000000000428.

5 Takahashi H, Yoshida T, Matsumoto T, Kameda Y, Takano Y, Tazo Y, et al. Frequent β-catenin gene mutations in atypical polypoid adenomyoma of the uterus. Hum Pathol 2014;45:33−40. https://doi.org/10.1016/j.humpath.2013.06.020.

6 Piscuoglio S, Burke KA, Ng CK, Papanastasiou AD, Geyer FC, Macedo GS, et al. Uterine adenosarcomas are mesenchymal neoplasms. J Pathol 2016;238:381−8. https://doi.org/10.1002/path.4675.

7 Strickland KC, Yuan L, Quade BJ, Nucci MR, Howitt BE. Clinicopathological and immunohistochemical features of uterine adenomyomatous polyps. Hum Pathol 2019;84:239−45. https://doi.org/10.1016/j.humpath.2018.10.002.

8 Heikkinen T, Äyräväinen A, Hänninen J, Ahvenainen T, Bützow R, Pasanen A, et al. MED12 mutations and fumarate hydratase inactivation in uterine adenomyomas. Hum Reprod Open 2018;2018:hoy020. https://doi.org/10.1093/hropen/hoy020.

9 Wang H, Ye J, Qian H, Zhou R, Jiang J, Ye L. High-resolution melting analysis of MED12 mutations in uterine leiomyomas in Chinese patients. Genet Test Mol Biomarkers 2015;19:162−6. https://doi.org/10.1089/gtmb.2014.0273.

10 Clark AD, Oldenbroek M, Boyer TG. Mediator kinase module and human tumorigenesis. Crit Rev Biochem Mol Biol 2015;50:393−426.

11 Mehine M, Mäkinen N, Heinonen HR, Aaltonen LA, Vahteristo P. Genomics of uterine leiomyomas: insights from high-throughput sequencing. Fertil Steril 2014;102:621−9. https://doi.org/10.1016/j.fertnstert.2014.06.050.

12 Li Z, Maeda D, Kudo-Asabe Y, Tamura D, Nanjo H, Hayashi A, et al. MED12 is frequently mutated in ovarian and other adnexal leiomyomas. Hum Pathol 2018;81:89−95. https://doi.org/10.1016/j.humpath.2018.06.013.

13 Schwetye KE, Pfeifer JD, Duncavage EJ. MED12 exon 2 mutations in uterine and extrauterine smooth muscle tumors. Hum Pathol 2014;45:65−70. https://doi.org/10.1016/j.humpath.2013.08.005.

14 Lien HC, Huang CS, Yang YW, Jeng YM. Mutational analysis of MED12 exon 2 in a spectrum of fibroepithelial tumours of the breast: implications for pathogenesis and histogenesis. Histopathology 2016;68:433−41. https://doi.org/10.1111/his.12764.

15 Lim WK, Ong CK, Tan J, Thike AA, Ng CC, Rajasegaran V, et al. Exome sequencing identifies highly recurrent MED12 somatic mutations in breast fibroadenoma. Nat Genet 2014;46:877−80. https://doi.org/10.1038/ng.3037.

16 Mishima C, Kagara N, Tanei T, Naoi Y, Shimoda M, Shimomura A, et al. Mutational analysis of MED12 in fibroadenomas and phyllodes tumors of the breast by means of targeted next-generation sequencing. Breast Cancer Res Treat 2015;152:305−12. https://doi.org/10.1007/s10549-015-3469-1.

17 Yoshida M, Sekine S, Ogawa R, Yoshida H, Maeshima A, Kanai Y, et al. Frequent MED12 mutations in phyllodes tumours of the breast. Br J Cancer 2015;112:1703−8. https://doi.org/10.1038/bjc.2015.116.

18 Zaloudek CJ, Hendrickson MR, Soslow RA. Mesencymal tumors of the uterus. In: Kurman RJ, Ellenson LH, Ronnett BM, editors. Blaustein's Pathology of the Female Genital Tract. Boston: Springer; 2011. p. 453−527.

19 Mäkinen N, Mehine M, Tolvanen J, Kaasinen E, Li Y, Lehtonen HJ, et al. MED12, the mediator complex subunit 12 gene, is mutated at high frequency in uterine leiomyomas. Science 2011;334:252−5. https://doi.org/10.1126/science.1208930.

20 Matsubara A, Sekine S, Yoshida M, Yoshida A, Taniguchi H, Kushima R, et al. Prevalence of MED12 mutations in uterine and extrauterine smooth muscle tumours. Histopathology 2013;62:657−61. https://doi.org/10.1111/his.12039.

21 Heinonen HR, Sarvilinna NS, Sjöberg J, Kämpjärvi K, Pitkänen E, Vahteristo P, et al. MED12 mutation frequency in unselected sporadic uterine leiomyomas. Fertil Steril 2014;102:1137−42. https://doi.org/10.1016/j.fertnstert.2014.06.040.

22 Inoue S, Hirota Y, Ueno T, Fukui Y, Yoshida E, Hayashi T, et al. Uterine adenomyosis is an oligoclonal disorder associated with KRAS mutations. Nat Commun 2019;10:5785. https://doi.org/10.1038/s41467.019.13708.y.

23 Mehine M, Kaasinen E, Mäkinen N, Katainen R, Kämpjärvi K, Pitkänen E, et al. Characterization of uterine leiomyomas by whole-genome sequencing. N Engl J Med 2013;369:43−53. https://doi.org/10.1056/NEJMoa1302736.

24 Mehine M, Kaasinen E, Heinonen HR, Mäkinen N, Kämpjärvi K, Sarvilinna N, et al. Integrated data analysis reveals uterine leiomyoma subtypes with distinct driver pathways and biomarkers. Proc Natl Acad Sci U S A 2016;113:1315−20. https://doi.org/10.1073/pnas.1518752113.

25 Mäkinen N, Kämpjärvi K, Frizzell N, Bützow R, Vahteristo P. Characterization of MED12, HMGA2, and FH alterations reveals molecular variability in uterine smooth muscle tumors. Mol Cancer 2017;16:101. https://doi.org/10.1016/j.ejmg.2019.103768.

26 Kämpjärvi K, Mäkinen N, Kilpivaara O, Arola J, Heinonen HR, Böhm J, et al. Somatic MED12 mutations in uterine leiomyosarcoma and colorectal cancer. Br J Cancer 2012;107:1761−5. https://doi.org/10.1038/j.bjc.2012.428.

27 Markowski DN, Bartnitzke S, Löning T, Drieschner N, Helmke BM, Bullerdiek J. MED12 mutations in uterine fibroids−their relationship to cytogenetic subgroups. Int J Cancer 2012;131:1528−36. https://doi.org/10.1002/ijc.428.27424.