

Mutations of *RAS* genes in endometrial polyps

TAKASHI TAKEDA^{1*}, KOUJI BANNO^{1*}, YUSUKE KOBAYASHI¹, MASATAKA ADACHI¹,
MEGUMI YANOKURA¹, EIICHIRO TOMINAGA¹, KENJIRO KOSAKI² and DAISUKE AOKI¹

¹Department of Obstetrics and Gynecology, ²Center for Medical Genetics,
Keio University School of Medicine, Tokyo 160-8582, Japan

Received May 31, 2019; Accepted August 27, 2019

DOI: 10.3892/or.2019.7353

Abstract. Endometrial polyps are common, yet the molecular mechanisms underlying their formation and progression remain unclear. We examined gene mutations possibly related to the pathogenesis of endometrial polyps, as well as to their clinical features. Four premenopausal patients with endometrial polyps, who were not under drug treatment, were recruited. Whole exomes of endometrial polyps and peripheral blood lymphocytes were analyzed by next-generation sequencing, and somatic mutations were derived by subtraction. Then, 35 samples of endometrial polyps and 12 samples of atypical polypoid adenomyoma were newly recruited to validate the identified mutations by polymerase chain reaction-reverse sequence specific oligonucleotide method. The mutations were also analyzed in separate stromal and glandular components of the polyps after laser-capture microdissection. Whole exome sequencing revealed that *KRAS* mutations were the only type of mutation detectable in multiple cases (2/4). Targeted mutation analysis revealed that 16 of 35 samples (45.7%) of endometrial polyps harbored *RAS* mutations. Mutation-positive cases exhibited a significantly higher number of endometrial polyps (3.25 ± 2.70 vs. 1.74 ± 0.87 , $P=0.045$). Laser-capture microdissection in *NRAS*-mutated endometrial polyps revealed that both stromal and glandular components harbored *RAS* mutations. There was no *RAS* mutation in 12 samples of atypical polypoid adenomyoma. This is the first report demonstrating that pathogenic *RAS* mutations are frequent in non-treated endometrial polyps. *RAS* mutations may have an important role in tumorigenesis and in the formation of multiple endometrial polyps.

Introduction

An endometrial polyp is defined as a localized, disorganized proliferation of benign glandular and stromal elements protruding from the surface of the endometrium. Polyps are common and can occur at any age but are mostly observed in the perimenopausal period (1). Endometrial polyps are usually asymptomatic and found incidentally; however, they sometimes cause abnormal uterine bleeding and infertility. They arise as monoclonal overgrowths of genetically altered endometrial stromal cells with secondary induction of polyclonal benign glands (2). Tamoxifen and estrogen exposure are known to increase the frequency and number of endometrial polyps, and several studies have shown that body mass index, waist circumference, and insulin resistance are related to the presence of these polyps (3,4). The relationship of tamoxifen with endometrial polyps has been widely investigated, and it has been suggested that tamoxifen may induce *KRAS* mutations in endometrial cells and cause the recurrence of endometrial polyps and cancer (5,6). Several studies have also investigated the relationships of endometrial polyps with gene expression, mutation, and the immune system (7-10). However, the pathogenesis of endometrial polyps is still debated.

Next-generation sequencing has produced a breakthrough in genomic research and has facilitated mutational analysis by whole exome sequencing (11). To explore cancer genomic alterations, a large number of cancer genomes have been sequenced worldwide, resulting in the implementation of projects such as The Cancer Genome Atlas (TCGA) and The International Cancer Genome Consortium (ICGC). Whole exome sequencing has been the main platform for these sequencing initiatives, and data on the mutation of protein-coding regions have been accumulated in relation to all types of cancers (12,13).

In this study, we performed whole exome sequencing and targeted mutation analysis to verify the presence of somatic mutations in benign endometrial polyps and identify the relevant driver mutations. Our results indicated the importance of oncogenic mutation in benign endometrial polyps and may lead to a new discovery in the tumorigenesis of endometrial tumors.

Materials and methods

Patient selection and tissue samples for whole exome sequencing. After approval of the Ethics Committee of Keio University

Correspondence to: Dr Kouji Banno, Department of Obstetrics and Gynecology, Keio University School of Medicine, 35 Shinanomachi, Shinjyuku-ku, Tokyo 160-8582, Japan
E-mail: kbanno@z7.keio.jp

*Contributed equally

Key words: endometrial polyp, *RAS* mutation, tumorigenesis, atypical polypoid adenomyoma, benign tumors, endometrial cancer

School of Medicine (No. 2015-0032) and provision of written informed consent, four patients were recruited for whole exome analysis. These patients were scheduled for transcervical resection of endometrial polyps due to their symptoms (infertility, abnormal uterine bleeding). They were premenopausal and not taking any drugs that might affect the endometrium (e.g. oral contraceptives, tamoxifen). Endometrial polyps were removed by transcervical resection, and peripheral blood lymphocytes were collected as control samples. One polyp was randomly selected for analysis in patients with multiple polyps. These polyps were dissected for analysis and pathological assessment. Samples were stained with hematoxylin and eosin, and those containing about 20-50% of stromal components were selected. Two independent pathologists confirmed the diagnosis of endometrial polyp. Clinical data (age, body mass index, medical history) were obtained from the clinical records. All patients received an outpatient hysteroscopy prior to transcervical resection. The endometrial polyps were counted by two independent physicians who were blinded to information on gene analysis. Specifically, one physician counted the polyps at the time of outpatient hysteroscopy and another physician counted them by reviewing the recorded videos and photos of the hysteroscopy.

Whole exome sequencing. DNA was extracted from endometrial polyps and peripheral blood lymphocytes, and whole exome sequencing was performed using a next-generation sequencer HiSeq 2500 (Illumina, San Diego, CA, USA) according to the manufacturer's protocol and previous reports (14-16). In short, a sequencing library was built using a SureSelect XT Human All Exon kit and sequencing analysis was performed using a HiSeq 2500 system with HiSeq Rapid SBS Kit v2-HS (Illumina) according to the manufacturer's instructions. The sequencing quality was evaluated by a bio-analysis company (Takara Bio Inc., Shiga, Japan). Polyp-specific somatic mutations were derived by subtracting lymphocyte sequencing data from those of the polyps.

Confirmation of observed RAS mutation. We focused on one somatic mutation that was found in endometrial polyps. Validation was performed by targeted mutation analysis via the polymerase chain reaction-reverse sequence-specific oligonucleotide (PCR-rSSO) method. Specifically, 34 new patients were recruited by the same selection criteria that had been used for whole exome sequencing (endometrial polyp, premenopausal, no medication). Patient recruitment was approved by the Ethics Committee of Keio University School of Medicine (No. 2007-0081) and written informed consent was obtained from all participating patients. Tissue samples from endometrial polyps were formalin-fixed and paraffin-embedded (FFPE), and were diagnosed by two independent pathologists. A total of 35 samples were collected from the 34 patients, including one recurrence of endometrial polyps. DNA extraction and mutation analysis for RAS genes were conducted at LSI Medience Corp. (Tokyo, Japan). Genomic DNA was extracted from tissue and serial slices of 7 μ m were prepared. After deparaffinization with xylene, the tissue sections were stained with hematoxylin and eosin, and the target lesions were dissected for analysis. PCR-rSSO with a Mebgen™ Rasket Kit (Medical and Biological Laboratories Co., Nagoya, Japan) was performed for RAS mutation analysis.

This kit can detect 48 mutation hotspots of RAS (*KRAS* and *NRAS*) and is used clinically to detect RAS mutations in colorectal cancer as biomarkers of unfavorable response to the anti-EGFR antibody (17).

Laser-capture microdissection and RAS-targeted sequencing. We analyzed the RAS mutations in stromal and glandular components of endometrial polyps using laser-capture microdissection. An *NRAS*-mutated case was selected based on the presence of an adequate DNA quantity from each component. The RAS mutation was analyzed in separate glandular and stromal components. Serial slices of 7 μ m were prepared from a block. After deparaffinization with xylene, the tissue sections were stained with hematoxylin and eosin, and the glandular and stromal lesions were dissected by a PALM-IV system (Carl Zeiss Microscopy, Oberkochen, Germany). All glandular lesions were microdissected and collected, and the remaining regions on the slide were regarded as stromal lesions. Each sample was placed in a 200- μ l microtube and DNA was extracted using a NucleoSpin DNA FFPE XS kit (Takara Bio, Inc.). The *NRAS* exon 2 was amplified from the extracted DNA by Applied Biosystems AmpliTaq Gold DNA polymerase (Thermo Fisher Scientific, Inc., Waltham, MA, USA) with the *NRAS* exon2 primer (Table SI). PCR products were purified using a NucleoSpin Gel and PCR Clean-Up Kit (Takara Bio) and then ligated to a pGEM-T easy vector (Promega, Fitchburg, WI, USA), followed by transformation of Sure2 competent cells (Agilent, Santa Clara, CA, USA). Competent cells were cultured on LB plates with ampicillin, and 30 colonies were chosen for each sample by color selection. The inserted DNA was amplified from each colony using SP6 and T7 primers (Table SI) and PCR products were analyzed by Sanger sequencing using the same *NRAS* exon2 primer pair.

Additional RAS mutation analysis for atypical polypoid adenomyoma. The RAS mutation was also investigated in atypical polypoid adenomyoma (APAM) by the PCR-rSSO method used in the analysis of endometrial polyps. Ten patients with APAM without premalignant or malignant components were recruited and provided written informed consent. In total, 12 samples from 10 patients were analyzed. APAM samples were obtained from two patients by diagnostic biopsy or resection and therapeutic hysterectomy. The pathological diagnosis was confirmed by two independent pathologists.

Statistical analysis. The clinical findings were compared by t-test and Pearson's Chi-squared test using Graph Pad Prism 7 (GraphPad Software, La Jolla, CA, USA), with the significance threshold set at $P < 0.05$.

Results

Whole exome analysis of endometrial polyps. Whole exome sequencing was performed on samples from 4 patients using DNA isolated from endometrial polyps and peripheral blood lymphocytes. We identified 22 nonsynonymous somatic mutations, including 21 missense and 1 nonsense mutations in endometrial polyps. No indel, frameshift or synonymous mutations were detected. The number of mutations varied from 2 to 10 per patient. Two of 4 endometrial polyps harbored *KRAS*

Table I. Results of the whole exome sequencing for 4 cases of endometrial polyps.

Case	Age (years)	BMI (kg/m ²)	Parity	Genes derived from NGS
1	35	21.3	G0P0	<i>STIL</i> (c.3425C>G, p.P1142R) <i>ICAI1L</i> (c.6T>G, p.D2E) <i>NT5E</i> (c.1060C>T, p.R354C) <i>KRAS</i> (c.37G>T, p.G13C) <i>SRSF9</i> (c.293G>T, p.R98L) <i>CNTNAP4</i> (c.2225C>T, p.A742V) <i>PPP2R1A</i> (c.767C>A, p.S256Y)
2	36	21.9	G0P0	<i>PPP1R12B</i> (c.2444A>T, p.K815M) <i>RAD21</i> (c.428T>C, p.I143T)
3	36	24.4	G1P1	<i>HECTD3</i> (c.1711C>T, p.R571C) <i>RAP1GAP2</i> (c.1243C>T, p.P415S) <i>ARHGAP35</i> (c.1190T>C, p.M397T) <i>MAGEA4</i> (c.616G>A, p.V206I)
4	35	21.0	G0P0	<i>MORNI</i> (c.254C>T, p.T85I) <i>DZIP3</i> (c.2587G>A, p.E863K) <i>SCUBE2</i> (c.1724G>C, p.R575P) <i>KRAS</i> (c.35G>T, p.G12V) <i>DHRS12</i> (c.488C>A, p.T163K) <i>YLPM1</i> (c.5183A>G, p.Y1728C) <i>TIMM21</i> (c.629C>T, p.A210V) <i>MAGEB2</i> (c.350C>A, p.S117*) <i>MED12</i> (c.3412C>G, p.R1138G)

NGS, next generation sequencing; BMI, body mass index.

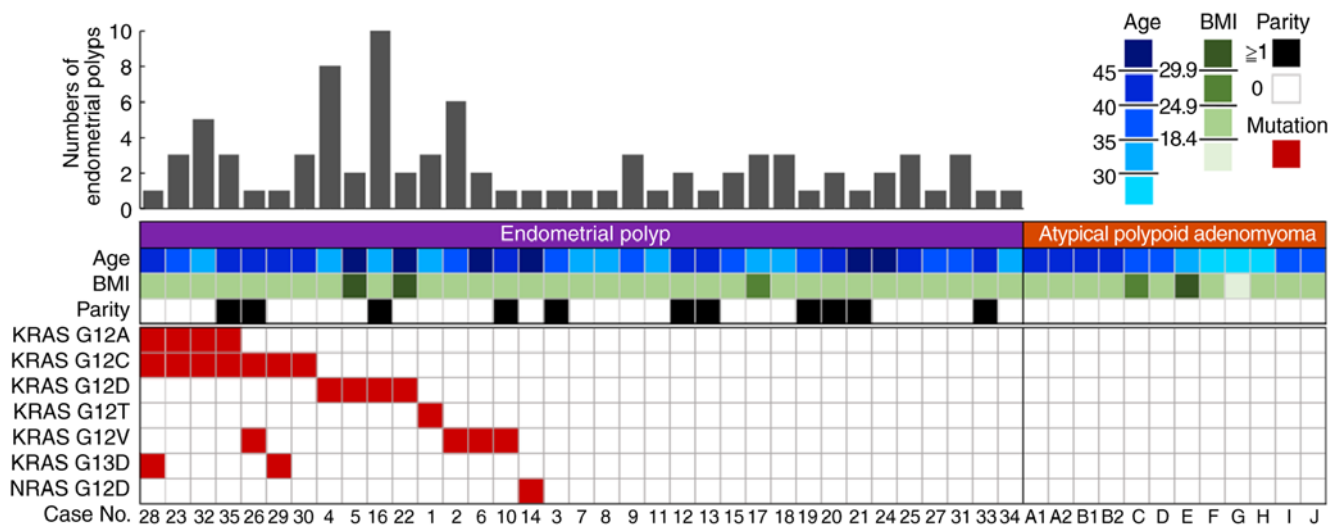


Figure 1. RAS mutation analysis of endometrial polyps and atypical polypoid adenomyoma. Thirty-five samples of endometrial polyps and 12 samples of atypical polypoid adenomyoma were analyzed for RAS mutations. Numbers of endometrial polyps, age, body mass index, parity, mutation data are summarized in the figure. Case 5 and 22 are samples from the same patient with recurrence of endometrial polyps (case 5: Primary, case 22: Recurrence).

mutations: c.37G>T (p.G13C) in patient 1 and c.35G>T (p.G12V) in patient 4. Additional mutations, including PPP2R1A (protein phosphatase 2 regulatory subunit α) and ARHGAP35 (Rho GTPase activating protein 35), were found (Tables I and SII).

RAS mutation analysis by polymerase chain reaction-reverse sequence-specific oligonucleotide method. RAS mutations were analyzed in 35 endometrial polyp samples from 34 patients (including one recurrent case) and 12 APAM samples from 10 patients by PCR-rSSO for validation. This analysis revealed that 16 of 35 cases (45.7%) of endometrial polyps harbored RAS mutations (15 KRAS, one NRAS); 10 cases had a single RAS mutation, and 6 had multiple RAS mutations, varying from 2 to 3 per endometrial polyp. The RAS mutations were all found in exon 2 of KRAS and NRAS (KRAS G12V, G12D, G12C, G12A, G13D; NRAS G12D). Two metachronous endometrial polyp samples (cases 5 and 22) from a patient with recurrence of endometrial polyps harbored the same mutation (KRAS G12D). There were no mutations

in the 12 atypical polypoid adenomyoma samples (Fig. 1). The comparison between 16 cases with RAS mutations and 19 cases without RAS mutations showed that the number of endometrial polyps was significantly higher in RAS-mutated cases, as assessed by outpatient hysteroscopy (3.25 ± 2.70 vs. 1.74 ± 0.87 , $P=0.045$). There were no differences in age, body mass index, and parity between these two groups (Table II).

RAS mutations are present in both stromal and glandular components of endometrial polyps. We next used laser-capture microdissection to investigate whether the RAS mutations were harbored by glandular or stromal components of endometrial polyps. Case 14, carrying the NRAS mutation, was selected for this analysis because it contained an adequate quantity of DNA from each cellular component. The single endometrial polyp was 25 mm in size and had developed from the fundus of the endometrial cavity. Sanger sequencing of the glandular and stromal components showed that both harbored an NRAS mutation (c.35C>A, p.G12D; Fig. 2).

Table II. Clinical characteristics of the cases with endometrial polyps with or without *RAS* mutation.

Clinical characteristics	<i>RAS</i> mutation		Others		P-value
Age (years)	40.9	±5.6	38.5	±4.7	0.165 ^a
Body mass index (kg/m ²)	21.9	±4.6	20.5	±2.8	0.258 ^a
Multigravida	4	(25.0%)	7	(36.8%)	0.493 ^b
Numbers of polyps	3.25	±2.70	1.74	±0.87	0.045 ^b

^at-test, ^bFisher exact test. P-value in bold indicates statistical significance. All the data shown in this table with ± represented the mean ± standard deviation.

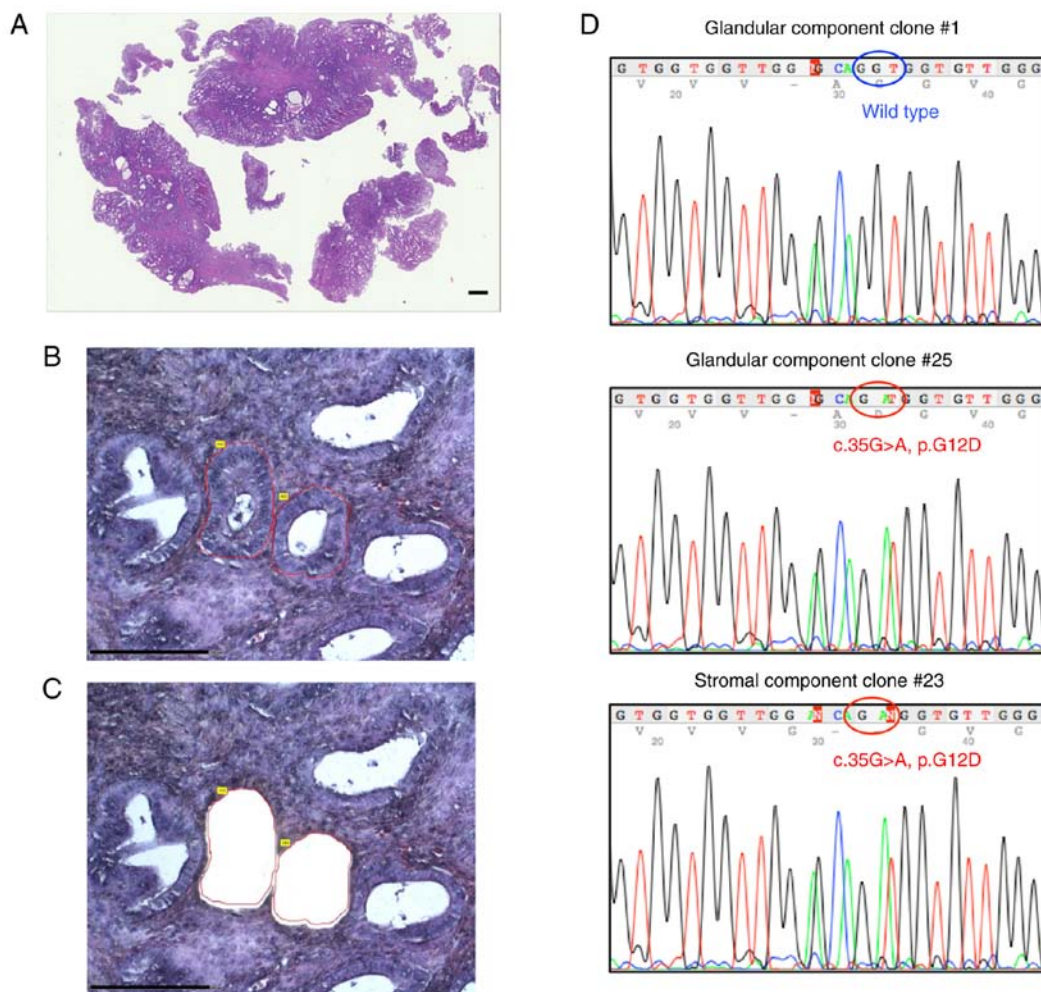


Figure 2. Laser-capture microdissection and *NRAS* gene sequencing. (A) An entire endometrial polyp with an *NRAS* mutation (c.35C>A, p.G12D). This polyp was analyzed for *NRAS*-targeted sequencing after laser-capture microdissection (original magnification, x4; scale bar, 1 mm). (B) Hematoxylin and eosin staining before laser-capture microdissection. (C) Hematoxylin and eosin staining after laser-capture microdissection (original magnification, x10; scale bar, 150 μ m). Red lines show the profile of laser-capture dissection; glandular components were dissected according to the target lines. Other glandular lesions were also dissected. The remaining lesions were collected as stromal lesions. (D) Result of *NRAS* sequencing. Sanger sequencing of glandular and stromal components collected from laser-capture microdissection showed that both components harbored an *NRAS* mutation (c.35G>A, p.G12D).

Discussion

In the present study, 45.7% of the examined endometrial polyps harbored *RAS* mutations. It is well known that endometrial cancers may contain *KRAS* mutations, with a reported rate of 10–30% (18–20). Furthermore, *KRAS* mutations were detected in 30–50% of *POLE* mutated (ultramutated) or microsatellite

instability (hypermuted) endometrial cancers (21,22). We found a surprisingly high frequency of *RAS* mutations in endometrial polyps, a type of lesion generally regarded as benign.

Endometrial polyps are monoclonal overgrowths of endometrial stromal cells with secondary induction of polyclonal benign glands (2). These non-atypical polyps usually do not develop into carcinoma, while colon polyps can sequentially

advance to colon carcinoma. *KRAS* mutations in colon polyps are correlated with the development of advanced polyps and adenomas. Moreover, *KRAS* mutations in colon polyps correlate with a larger lesion size and a higher number of polyps, and may be a useful marker for predicting the development of metachronous advanced neoplasia (23-25). In contrast, the pathogenesis of endometrial polyps is not well known, except for the fact that tamoxifen and estrogen exposure are risk factors. *KRAS* mutations in endometrial polyps have been found in elderly, tamoxifen-treated patients (5,6). We investigated endometrial polyps from premenopausal, drug-free patients to exclude age and artificial hormonal factors. In this study, several cases carried multiple *RAS* mutations, indicating that these events may favor the development of endometrial polyps.

The association between oncogenic mutations and benign tumors has been extensively investigated, and several oncogenes have been reported in benign conditions (26). Recent reports revealed that 26% of cases of deep infiltrating endometriosis harbor somatic cancer driver mutations (*KRAS*, *PIK3CA*, *ARID1A*, *PPP2R1A*) in glandular, but not stromal, compartments of deep endometriotic lesions (27,28). Furthermore, *KRAS*, *ARHGAP35*, and *PPP2R1A* mutations, which were detected by whole exome sequencing in this study, were recently found in uterine endometrial epithelium (29). Our result added a new information that *RAS* mutation in endometrial polyps has an important role in their multiple development.

Atypical polypoid adenomyoma (APAM) is defined as a mixture of polypoid lesion consisting of glands with cytological atypia and fibromuscular stroma (2). APAM is generally regarded as a benign lesion, however it frequently recurs and often coexists with atypical endometrial hyperplasia and endometrial adenocarcinoma (30). Therefore, we expected that APAM harbors *RAS* mutation more frequently than endometrial polyps. Previous small-scale studies have shown that APAM is associated with *MLH1* hypermethylation, and microdissected glandular components were shown to contain *CTNNB1* mutations (31,32). In addition, *KRAS* mutation was found in 4/16 cases (25%) without *BRAF* mutation (33), in contrast with the absence of *RAS* mutations in our APAM samples (0/12). Larger studies employing laser-microdissection followed by *RAS* mutation analysis in individual APAM components, will be necessary to draw final conclusions on this issue.

In conclusion, we found that *RAS* genes were frequently mutated in endometrial polyps, as assessed by whole exome sequencing and targeted mutation analysis. This is the first report showing a high frequency of pathogenic *RAS* mutations in non-treated endometrial polyps. *RAS* mutations may have an important role in tumorigenesis and in the formation of multiple endometrial polyps.

Acknowledgements

Not applicable.

Funding

This work was supported by the Japan Society for the Promotion of Science (JSPS) through a Grant-in-Aid for

Scientific Research (C) (16K11154), and by the Keio Gijuku Academic Development Fund. The funders had no role in the study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the manuscript for publication.

Availability of data and materials

The data analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

The concept was designed by TT, KB and KK. draft preparation, writing, and editing of the manuscript were undertaken by TT and KB. Molecular analyses were performed by TT, KB, MA and MY. Clinical procedures and sample collection were carried out by TT, KB, YK, MA, ET and DA. TT, KB and DA prepared the revised manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The Ethics Committees of Keio University School of Medicine approved the study. All the participants signed informed consent prior to participation.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' information

ORCID ID for authors: TT, 0000-0001-6686-5742; KB, 0000-0003-2610-2833; YK, 0000-0002-4503-2845; MA, 0000-0003-2974-8795; MY, 0000-0002-1505-3194; ET, 0000-0001-9243-0267; KK, 0000-0002-6798-8151; DA, 0000-0002-9596-8326.

References

1. Dreisler E, Stampe Sorensen S, Ibsen PH and Lose G: Prevalence of endometrial polyps and abnormal uterine bleeding in a Danish population aged 20-74 years. *Ultrasound Obstet Gynecol* 33: 102-108, 2009.
2. Kurman RJ, Carcangiu ML, Herrington CS and Young RH: WHO Classification of Tumours of Female Reproductive Organs, 4th edition. WHO Classification of Tumours. Vol 6. World Health Organization, Lyon, 2014.
3. Özkan NT, Tokmak A, Güzel Aİ, Özkan S and Çiçek MN: The association between endometrial polyps and metabolic syndrome: A case-control study. *Aust N Z J Obstet Gynaecol* 55: 274-278, 2015.
4. Serhat E, Cogendez E, Selcuk S, Asoglu MR, Arioglu PF and Eren S: Is there a relationship between endometrial polyps and obesity, diabetes mellitus, hypertension? *Arch Gynecol Obstet* 290: 937-941, 2014.

5. Tsujioka H, Hachisuga T, Fukuoka M, Ueda T, Miyahara D, Horiuchi S, Shiota K, Yoshizato T, Emoto M, Miyamoto S and Kawarabayashi T: Monitoring of endometrial K-ras mutation in tamoxifen-treated patients with breast cancer. *Int J Gynecol Cancer* 19: 1052-1056, 2009.
6. Tsujioka H, Hachisuga T, Hikita S, Ueda T, Yotsumoto F, Shiota K, Yoshizato T, Kawarabayashi T, Kuroki M and Miyamoto S: Apoptosis as a possible candidate mechanism for removal of tamoxifen-related endometrial cells with KRAS mutations. *Anticancer Res* 30: 3119-3123, 2010.
7. Dal Cin P, Wanschura S, Kazmierczak B, Tallini G, Dei Tos A, Bullerdiel J, Van den Berghe I, Moerman P and Van den Berghe H: Amplification and expression of the HMGIC gene in a benign endometrial polyp. *Genes Chromosomes Cancer* 22: 95-99, 1998.
8. Indraccolo U, Di Iorio R, Matteo M, Corona G, Greco P and Indraccolo SR: The pathogenesis of endometrial polyps: A systematic semi-quantitative review. *Eur J Gynaecol Oncol* 34: 5-22, 2013.
9. Zhu Y, Du M, Yi L, Liu Z, Gong G and Tang X: CD4⁺ T cell imbalance is associated with recurrent endometrial polyps. *Clin Exp Pharmacol Physiol* 45: 507-513, 2018.
10. Rios SS, Andrade RV, Pereira RW, Wall NR, Bahjri K, Caldas É, Cavalcante L and Figueiredo F: Microsatellite instability in endometrial polyps. *Eur J Obstet Gynecol Reprod Biol* 153: 193-197, 2010.
11. Sikkema-Raddatz B, Johansson LF, de Boer EN, Almomani R, Boven LG, van den Berg MP, van Spaendonck-Zwarts KY, van Tintelen JP, Sijmons RH, Jongbloed JD and Sinke RJ: Targeted next-generation sequencing can replace Sanger sequencing in clinical diagnostics. *Hum Mutat* 34: 1035-1042, 2013.
12. Forbes SA, Beare D, Gunasekaran P, Leung K, Bindal N, Boutselakis H, Ding M, Bamford S, Cole C, Ward S, *et al*: COSMIC: Exploring the world's knowledge of somatic mutations in human cancer. *Nucleic Acids Res* 43 (Database Issue): D805-D811, 2015.
13. Nakagawa H and Fujita M: Whole genome sequencing analysis for cancer genomics and precision medicine. *Cancer Sci* 109: 513-522, 2018.
14. Ng SB, Buckingham KJ, Lee C, Bigham AW, Tabor HK, Dent KM, Huff CD, Shannon PT, Jabs EW, Nickerson DA, *et al*: Exome sequencing identifies the cause of a mendelian disorder. *Nat Genet* 42: 30-35, 2010.
15. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bembien LA, Berka J, Braverman MS, Chen YJ, Chen Z, *et al*: Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 437: 376-380, 2005.
16. Bentley DR, Balasubramanian S, Swerdlow HP, Smith GP, Milton J, Brown CG, Hall KP, Evers DJ, Barnes CL, Bignell HR, *et al*: Accurate whole human genome sequencing using reversible terminator chemistry. *Nature* 456: 53-59, 2008.
17. Yoshino T, Muro K, Yamaguchi K, Nishina T, Denda T, Kudo T, Okamoto W, Taniguchi H, Akagi K, Kajiura T, *et al*: Clinical validation of a multiplex kit for RAS mutations in colorectal cancer: Results of the RASKET (RAS KEy Testing) prospective, multicenter study. *EBioMedicine* 2: 317-323, 2015.
18. Pappa KI, Choleza M, Markaki S, Giannikaki E, Kyrouti A, Vlachos G, Voulgaris Z and Anagnostou NP: Consistent absence of BRAF mutations in cervical and endometrial cancer despite KRAS mutation status. *Gynecol Oncol* 100: 596-600, 2006.
19. Garcia-Dios DA, Lambrechts D, Coenegrachts L, Vandenput I, Capoen A, Webb PM, Ferguson K, ANECS, Akslen LA, Claes B, *et al*: High-throughput interrogation of PIK3CA, PTEN, KRAS, FBXW7 and TP53 mutations in primary endometrial carcinoma. *Gynecol Oncol* 128: 327-334, 2013.
20. van der Putten LJM, van Hoof R, Tops BBJ, Snijders MPLM, van den Berg-van Erp SH, van der Wurff AAM, Bulten J, Pijnenborg JMA and Massuger LFAG: Molecular profiles of benign and (pre)malignant endometrial lesions. *Carcinogenesis* 38: 329-335, 2017.
21. Cancer Genome Atlas Research Network, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, Robertson AG, Pashtan I, Shen R, *et al*: Integrated genomic characterization of endometrial carcinoma. *Nature* 497: 67-73, 2013.
22. Yeramian A, Moreno-Bueno G, Dolcet X, Catusas L, Abal M, Colas E, Reventos J, Palacios J, Prat J and Matias-Guiu X: Endometrial carcinoma: Molecular alterations involved in tumor development and progression. *Oncogene* 32: 403-413, 2013.
23. Lorentzen JA, Grzyb K, De Angelis PM, Hoff G, Eide TJ and Andresen PA: Oncogene mutations in colorectal polyps identified in the norwegian colorectal cancer prevention (NORCCAP) screening study. *Clin Med Insights Pathol* 9: 19-28, 2016.
24. Juárez M, Egoavil C, Rodríguez-Soler M, Hernández-Illán E, Guarinos C, García-Martínez A, Alenda C, Giner-Calabuig M, Murcia O, Mangas C, *et al*: KRAS and BRAF somatic mutations in colonic polyps and the risk of metachronous neoplasia. *PLoS One* 12: e0184937, 2017.
25. Yamane LS, Scapulatempo-Neto C, Alvarenga L, Oliveira CZ, Berardinelli GN, Almodova E, Cunha TR, Fava G, Colaiacovo W, Melani A, *et al*: KRAS and BRAF mutations and MSI status in precursor lesions of colorectal cancer detected by colonoscopy. *Oncol Rep* 32: 1419-1426, 2014.
26. Kato S, Lippman SM, Flaherty KT and Kurzrock R: The conundrum of genetic 'Drivers' in benign conditions. *J Natl Cancer Inst* 108: pii: djw036, 2016.
27. Anglesio MS, Papadopoulos N, Ayhan A, Nazeran TM, Noë M, Horlings HM, Lum A, Jones S, Senz J, Seckin T, *et al*: Cancer-associated mutations in endometriosis without cancer. *N Engl J Med* 376: 1835-1848, 2017.
28. Noe M, Ayhan A, Wang TL and Shih IM: Independent development of endometrial epithelium and stroma within the same endometriosis. *J Pathol* 245: 265-269, 2018.
29. Suda K, Nakaoka H, Yoshihara K, Ishiguro T, Tamura R, Mori Y, Yamawaki K, Adachi S, Takahashi T, Kase H, *et al*: Clonal expansion and diversification of cancer-associated mutations in endometriosis and normal endometrium. *Cell Rep* 24: 1777-1789, 2018.
30. Heatley MK: Atypical polypoid adenomyoma: A systematic review of the English literature. *Histopathology* 48: 609-610, 2006.
31. Ota S, Catusas L, Matias-Guiu X, Bussaglia E, Lagarda H, Pons C, Muñoz J, Kamura T and Prat J: Molecular pathology of atypical polypoid adenomyoma of the uterus. *Hum Pathol* 34: 784-788, 2003.
32. Takahashi H, Yoshida T, Matsumoto T, Kameda Y, Takano Y, Tazo Y, Inoue H and Saegusa M: Frequent β -catenin gene mutations in atypical polypoid adenomyoma of the uterus. *Hum Pathol* 45: 33-40, 2014.
33. Němejcová K, Kenny SL, Laco J, Škapa P, Staněk L, Zikán M, Kleiblová P, McCluggage WG and Dundr P: Atypical polypoid adenomyoma of the uterus: An immunohistochemical and molecular study of 21 Cases. *Am J Surg Pathol* 39: 1148-1155, 2015.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.