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Research Paper

Analysis of *MED12* Mutation in Multiple Uterine Leiomyomas in South Korean patients

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Abstract

Uterine leiomyomas are one of the most common benign gynecologic tumors, but the exact causes are not completely understood. In 2011, through DNA sequencing, *MED12* mutation was discovered in approximately 71% of uterine leiomyomas. Several recent studies confirmed the high frequency of *MED12* mutation in uterine leiomyoma. Nevertheless, no study has been done on *MED12* mutation in the case of patients with multiple leiomyomas in a patient. The purpose of this study was to investigate the frequency of *MED12* mutations in uterine leiomyomas of South Korean patients. In addition, we examined *MED12* mutation in multiple leiomyomas in the same patients.

Uterine leiomyoma tissues were obtained from symptomatic women who underwent hysterectomy or myomectomy for medically indicated reasons. We collected 60 uterine leiomyomas from 41 women. Tumor size ranged from 1 to 12cm. Patients' ages ranged from 25 to 55 years with an average of 38.4 years.

Of the 60 tumors, 40 (66.67%) displayed *MED12* mutation. Among the 41 patients, 14 patients had multiple leiomyomas and we analyzed those multiple leiomyomas. Three of them had the same mutations. Five of them, each leiomyoma had a different mutation. Two of them did not have mutation. Four of them had both mutation-positive and mutation-negative leiomyomas.

In conclusion, we confirmed the high frequency of the *MED12* mutation in uterine leiomyomas of South Korean patients. We also identified various *MED12* mutation status in patients with multiple leiomyomas. This suggests that in a given patient, different tumors may have arisen from different cell origins and therefore it is supposed that occurrence of multiple leiomyoma in a single patient may not be caused by intrauterine metastasis or dissemination.

Key words: *MED12*, uterine leiomyoma, somatic mutation, multiple leiomyoma.

Introduction

Uterine leiomyomas, also known as fibroids are the most common gynecological neoplasm in women of reproductive age. Recently, uterine leiomyomas are increasing in nulliparous women due to delayed marriage and delivery. It also causes female infertility, abnormal uterine bleeding, dysmenorrhea, and pelvic pain [1].

Unfortunately, the exact causes of uterine leiomyomas are not completely understood. Nulliparity, early menarche, late menopause and obesity increase the size of lesions while the tumors usually shrink after menopause, which supports estrogen and progesterone are important regulators of leiomyoma growth [2].

In recent studies, several chromosomal aberrations have been observed in approximately 40%–50% of uterine leiomyomas, such as deletions of 7q and rearrangements involving 12q15 and 6p21. These occur in approximately 17%, 20%, and 5% of karyotypically abnormal lesions, respectively [3, 4].

In addition to chromosomal change, Makinen et al. recently examined uterine leiomyoma tissues by exome sequencing and identified somatic mutations of mediator complex subunit 12 (*MED12*). They discovered mutations in *MED12* exon 2 in 159 of 225 uterine leiomyomas (71%). *MED12* is a subunit of the Mediator complex that regulates transcription via RNA polymerase II [5]. Both missense and inframe insertion-deletion mutations have been reported, with codon 44 being the most prevalent mutational hotspot (39%–96%) [6, 7]. The frequency of *MED12* mutations has been reported to vary 50% to 80%, depending on the ethnicity of examined patients [6, 8, 9].

In the study of Makinen et al. no tumor displayed more than one mutation [5]. And former studies did not describe the *MED12* mutation status of multiple leiomyoma in one patient. The purpose of this study was to investigate the frequency of *MED12* mutations in uterine leiomyoma of South Korean patients to confirm that *MED12* had a major role in the pathogenesis of uterine leiomyoma. In addition, we examined *MED12* mutation in multiple leiomyomas in the same patients.

Materials and methods

Tissue samples and DNA extraction

Uterine leiomyoma tissues were obtained from symptomatic women who underwent hysterectomy or myomectomy for medically indicated reasons at Seoul St. Mary's Hospital. We collected 60 uterine leiomyomas from 41 women. Tumor size ranged from 1 to 12 cm. Patients' age ranged from 25 to 55 years with an average of 38.4 years. The other basic characteristics of all enrolled patients are summarized in Table 1.

Table 1. Basic characteristics of the 41 enrolled patients

Variable	Data
Age	38.4 ± 7.2*
Body Mass Index	22.6 ± 9.0
Menarche	13.8 ± 1.3
Parity	0.63 ± 0.9
Married patients	19 (46.3%)**
Combined gynecologic disease	
Endometriosis	12 (29.1%)
Adenomyosis	5 (12.2%)

*mean ± SD; **number of patients (percentage)

Genomic DNA extraction

We used the Qiagen DNeasy Tissue kit (Qiagen, Hilden, Germany) and the protocols for fresh frozen tissues. Tissue samples were then lysed under denaturing conditions with a proteinase K digestion at 56°C for 3 h. DNA was purified by column purification with a filter membrane and stored in -20°C before use.

MED12 mutation analysis

10 ng of genomic DNA extracted from each leiomyoma tissues was amplified by polymerase chain reaction (PCR). Primers used for the amplification of *MED12* sequence were 5′-AACTAAACGCCGCTTTCCTG-3′ (forward) and 5′-TTCCTTCAGCCTGGCAGAG-3′(reverse); product size: 159 base pairs. The PCR products were electrophoresed in a 2% agarose gel. Isolated PCR products were sequenced directly by Big Dye Terminator v.3.1 Cycle sequencing chemistry on a 96 capillary array DNA sequencer ABI 3730XL (Applied Biosystems, Foster City, CA, USA).

Results

Of all 60 leiomyomas, 66.67% (40/60) had *MED12* mutations. All these point mutations were found in two nucleotide sites of c.130 and c.131. Six types of mutations were found and the most common mutation was c.130G>T (p.G44C) (Table 2).

Table 2. *MED 12* mutations in Uterine leiomyoma

MED12 mutation type	N=60
c.130G>A (p.G44S)	7
c.130G>C (p.G44R)	3
c.130G>T (p.G44C)	11
c.131G>A (p.G44D)	9
c.131G>C (p.G44A)	6
c.131G>T (p.G44V)	4
No mutation	20

Twenty-seven patients had single leiomyoma and average size of collected leiomyomas was 7.46 cm. Fourteen patients had multiple leiomyomas. All 33 leiomyomas from 14 patients showed average size of 4.21 cm which was significantly smaller than the mean size of single leiomyoma ($p < 0.05$). Of 27 patients who have only one leiomyoma, 40.74% showed no mutation while patients who have multiple leiomyoma showed no mutations in 27.27% (Table 3).

Table 3. Single versus multiple leiomyoma patient

Patients with single leiomyoma		Patients with multiple leiomyomas	
MED12 mutation type	N=27	*MED12* mutation type	N=33
c.130G>A (p.G44S)	2	c.130G>A (p.G44S)	5
c.130G>C (p.G44R)	3	c.130G>C (p.G44R)	0
c.130G>T (p.G44C)	4	c.130G>T (p.G44C)	7
c.131G>A (p.G44D)	3	c.131G>A (p.G44D)	6
c.131G>C (p.G44A)	4	c.131G>C (p.G44A)	2
c.131G>T (p.G44V)	0	c.131G>T (p.G44V)	4
No mutation	11 (40.74%)	No mutation	9 (27.27%)
Mean size (cm)	7.46	Mean size (cm)	4.21

We analyzed leiomyomas of patients who have multiple leiomyomas. Among 14 patients, mutations in each leiomyoma were identical in 3 patients (#13, #14, #34). And for 5 out of 14 patients, different mutations were found in each leiomyoma (#11, #36, #38, #39, #45). Two patients had no mutations in their leiomyomas (#33, #47). In 4 patients, some leiomyomas had mutations but other leiomyomas were mutation free (#21, #37, #48, #49) (Table 4).

Table 4. Various kinds of *MED12* mutations in multiple leiomyomas

Patients	Age	Size(cm)	Mutation status of MED12
#11	42	1	c.131G>T (p.G44V)
		3	c.130G>A (p.G44S)
#13	30	3	c.131G>A (p.G44D)
		6	c.131G>A (p.G44D)
#14	31	1	c.130G>T (p.G44C)
		3	c.130G>T (p.G44C)
#21	39	1	c.130G>A (p.G44S)
		1	No mutation
#33	39	2	No mutation
		8	No mutation
#34	25	1	c.130G>T (p.G44C)
		6	c.130G>T (p.G44C)
		9	c.130G>T (p.G44C)
#36	41	2	c.130G>A (p.G44S)
		6	c.131G>A (p.G44D)
#37	35	5	c.130G>T (p.G44C)
		8	No mutation
#38	33	2	c.131G>A (p.G44D)
		7	c.131G>C (p.G44A)
		11	c.131G>T (p.G44V)
#39	30	2	c.130G>T (p.G44C)
		6	c.131G>T (p.G44V)
#45	34	3	c.130G>A (p.G44S)
		7	c.131G>A (p.G44D)
#47	33	1	No mutation
		3	No mutation
		7	No mutation
#48	41	1	No mutation
		4	c.130G>A (p.G44S)
#49	40	2	No mutation
		4	c.131G>C (p.G44A)
		12	c.131G>T (p.G44V)

Discussion

MED12 mutation is the most common mutation in uterine leiomyomas. The mutation has been reported to vary from 40% to 85% depending on the ethnicity (Table 5). We examined the frequency of the *MED12* mutations in symptomatic South Korean patients. A total of 60 leiomyomas from 41 patients were studied, and 66.67% (40/60) harbored a *MED12* mutation. Comparing to the studies of Asian countries, this is higher than the frequency of previous Korean and Chinese studies and lower than that of the Japanese study [10-13].

In this study, multiple uterine leiomyomas seemed to have more *MED12* mutations than single uterine leiomyomas did (72.73% versus 59.26%), but there was no significant difference (p = 0.270). And the mean size of leiomyomas was smaller in patients with multiple leiomyomas significantly, which appears to be in correspondence with the outcome of previous study [6]. In 2015, Osinovskaya et al. demonstrated the difference of *MED12* mutation frequency between multiple and single uterine leiomyomas from 122 patients [14]. The frequency of *MED12* mutation was almost two-folds higher in the multiple uterine leiomyomas than in the single uterine leiomyomas, significantly (61% versus 32.5%, p = 0.003). However, they could not confirm the significant association between *MED12* mutation and tumor size. Therefore, larger sample size of study will be needed to evaluate the association between *MED12* mutation frequency and the number or the size of uterine leiomyoma.

Table 5. Frequency of the *MED12* stations in leiomyoma in various ethnicities.

Reference	Year	Nationality	Ethnicity	Frequency
Heinonen et al. [6]	2014	Finland	Caucasian	85.5% (65/76)
Makinen et al. [5]	2011	Finland	Caucasian	70.6% (159/225)
McGuire et al. [8]	2012	USA	Black American	78% (18/23)
			White American	66% (79/120)
Je et al. [10]	2012	Korea	Asian	52.2% (35/67)
Bertsch et al. [25]	2014	USA	Black women	79.0% (64/81)
			White women	71.6% (53/73)
			Hispanic women	81.3% (13/16)
			Asian women	66.7% (4/6)
Makinen et al. [9]	2011	South Africa	Black South African and Coloured	50% (14/28)
Matubara et al. [11]	2013	Japan	Asian	80% (36/45)
Ye et al. [12]	2015	China	Asian	54.39% (93/171)
Wu et al. [13]	2017	China	Asian	43.6% (158/362)
Osinovskaya et al. [14]	2016	Russia	Russian	51.5% (63/122)

Of 14 patients with multiple leiomyomas, 9 patients harbored different types of *MED12* mutations for each leiomyoma or had mutation-positive and -negative leiomyomas concurrently. Among the patients, 2 patients who had 3 masses showed different types respectively, which suggests that multiple leiomyomas may have arose from separate origin and they may not be caused by intrauterine metastasis or dissemination. The result shows that diverse factors may influence on the generation of leiomyoma and each lesion of multiple leiomyoma might have different genetic mutation. And we found some multiple leiomyomas had the same mutation types, which we could not exclude the possibility of concurrent same mutation. We considered sampling errors might have occurred but in the patient cases with multiple leiomyomas, we had extracted each tissue from definitely different tumors so we could

exclude the errors.

MED12 mutation is also detected in other uterine tumors such as leiomyosarcomas (30%) and smooth muscle tumor of uncertain malignant potential (8%) but not in other organs' tumors [15-17]. Je et al. reported that among 1,862 tumor tissues including a variety of carcinomas, leukemias and stromal tumors, 52.2% (35/67) of uterine leiomyomas and 0.3% (1/389) of colon carcinoma harbored *MED12* mutations [10]. Another study which examined uterine leiomyosarcoma and colorectal cancer showed similar results (7%, 0.5%) [18]. Interestingly, breast fibroadenoma harbored highly frequent *MED12* mutations [19]. No genes except *MED12* mutation were found in *MED12* mutation-positive and -negative leiomyomas by whole exome sequencing and this suggests that *MED12* mutation alone may be sufficient for leiomyoma tumorigenesis [20].

Recently, some studies have attempted to reveal the function of *MED12* mutation in leiomyoma pathogenesis. Di et al. examined *MED 12* mutation in uterine leiomyoma, myometrium and pseudocapsule. The mutation was harbored only in leiomyoma tissues. They also detected that high level of *IGF-2* mRNA when *MED12* missense mutations were expressed [21]. Kämpjärvi et al. examined exon 1 and exon 2 *MED12* mutations in total 611 samples of uterine leiomyosarcomas, extrauterine leiomyomas and leiomyosarcomas, endometrial polyps, and colorectal cancers. All of these tumors harbored both exon 1 and exon 2 mutations, despite significantly higher rates of exon 2 mutations. Also they observed that *MED12* mutations disrupt the interaction between MED12 and Cyclin C, CDK8/19 and interrupt the mediator-associated CDK kinase activity [22]. Previous studies have reported the interaction between *MED12* and β-catenin/Wnt pathway [5, 23]. However, according to Perot et al., there was no association between *MED12* mutations and β-catenin localization [24].

Throughout the study, we identified high frequency of the *MED12* mutation in uterine leiomyomas of South Korean patients. We also identified various *MED12* mutation status in multiple leiomyoma. This suggests that in a given patient, different tumors may have arisen from different cell origins and therefore it is supposed that occurrence of multiple leiomyoma in a single patient may not be caused by intrauterine metastasis or dissemination.

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Competing Interests

The authors have declared that no competing interest exists.

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