

Prevalence and clinical significance of mediator complex subunit 12 mutations in 362 Han Chinese samples with uterine leiomyoma

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Abstract. Uterine leiomyomas (ULs) are the most common gynecological benign tumors originating from the myometrium. Prevalent mutations in the mediator complex subunit 12 (MED12) gene have been identified in ULs, and functional evidence has revealed that these mutations may promote the development of ULs. However, whether MED12 mutations are associated with certain clinical characteristics in ULs remains largely unknown. In the present study, the potential mutations of MED12 and its paralogous gene, mediator complex subunit 12-like (MED12L), were screened in 362 UL tumors from Han Chinese patients. A total of 158 out of 362 UL tumors (43.6%) were identified as harboring MED12 somatic mutations, and the majority of these mutations were restricted to the 44th residue. MED12 mutations were also observed in 2 out of 145 (1.4%) adjacent control myometrium. Furthermore, the mutation spectrum of MED12 in the concurrent leiomyomas was noticeably different. Correlation analysis of MED12 mutations with the available clinical features indicated that patients with mutated MED12 tended to have smaller cervical diameters. By contrast, no MED12L mutation was identified in the present samples. In summary, the present study demonstrated the presence of prevalent MED12 somatic mutations in UL samples, and the MED12 mutation was associated with smaller cervical diameters. The low mutation frequency of MED12 in adjacent control myometrium indicated that MED12 mutation may be an early event in the pathogenesis of ULs.

Furthermore, MED12 mutation status in concurrent tumors from multiple leiomyomas supported several prior observations that the majority of these tumors arose independently.

Introduction

Uterine leiomyomas (ULs) are the most common gynecological benign tumors originating from the myometrium, affecting 60-80% of women of reproductive age (1), and resulting in abnormal uterine bleeding, recurrent miscarriage, pelvic pain, premature birth and infertility in 10-30% of cases (2-4). Clinically, ULs may grow as a single nodule or multiple concurrent lesions. At present, the most effective treatment strategy for ULs is a surgical operation, although certain medical drugs are also available, including gonadotropin-releasing hormone agonists and selective modulators of estrogen- and progesterone-receptors (5,6).

Heterozygous somatic mutations in exon 2 of the mediator complex subunit 12 (MED12) gene located on the X chromosome have been identified in 70.7% (159/225) of tumors with ULs (7). Subsequent studies using the same methods or direct Sanger sequencing have also identified prevalent MED12 mutations in ULs (8-12), breast fibroadenoma (13,14) and chronic lymphocytic leukemia (15), whereas MED12 mutations were less common in prostate (16,17) and colorectal cancer (10,18). Almost all identified MED12 mutations in ULs were heterozygous somatic mutations restricted to the 44th codon (p.G44) on exon 2, only occurring at low frequencies in adjacent codons, including codons 33, 36, 41 and 43 (7-9,11,12). MED12 belongs to the mediator complex and modulates RNA polymerase II activity, and thus is involved in global gene transcriptional regulation within cells (7,19). A previous *in vivo* study revealed that the MED12 p.G44D (c.131G>A) mutation (the most frequently observed mutation in ULs) may cause ULs and uterine hyperplasia in mice (20). Additional array comparative genomic hybridization analysis revealed that this mutation markedly increased genomic copy number gains and losses at the whole-genome scale, spanning multiple genes involved in cell cycle checkpoints and tumorigenesis (20).

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Accumulating evidence has confirmed frequent MED12 mutations in ULs (7-12). However, several issues regarding MED12 mutations in ULs remain unresolved. First, the clinical impacts of MED12 mutations in ULs remain scarce; only one study has reported that patients with MED12-mutated with ULs tend to harbor smaller sized leiomyomas compared with their counterparts without MED12 mutations (12). Second, it is largely unclear whether MED12 mutations also exist in the adjacent myometrium in patients with ULs. Third, the mutational status of MED12 in multiple concurrent leiomyomas within the same affected uterus remains unexplored in Han Chinese patients with ULs. Fourth, increasing evidence has suggested that paralogous genes are frequently mutated in certain cancer types (21-23), however, it is unknown whether MED12L (the paralogous gene of MED12) is also mutated in ULs.

To clarify these issues, MED12 and MED12L mutations were analyzed in 362 Han Chinese samples with ULs, and the results were compared with their clinical features. A high frequency (158/362, 43.6%) of MED12 mutations were identified in the samples used, and MED12 mutations were demonstrated to be associated with smaller cervical diameters. In addition, MED12 mutations occurred in the adjacent myometrium at a low frequency, and the mutational status of MED12 in concurrent leiomyomas from the same patients exhibited a noticeable difference. By contrast, no MED12L mutations were identified in the present sample set.

Materials and methods

Samples and clinical characteristics. A collection of 322 patients with ULs undergoing laparoscopic intracapsular myomectomy, including 362 tumors, 322 adjacent control myometrium and 322 paired peripheral blood lymphocytes, were recruited in the Department of Gynecology, Jiangxi Provincial Maternal and Child Health Hospital (Jiangxi, China) between March 2014 and February 2015. Among these patients, 290 patients had solitary leiomyomas and 32 patients had multiple leiomyomas (72 concurrent leiomyomas). All samples were diagnosed according to gynecological and abdominal ultrasonography examinations, and each patient provided informed consent. The present study conformed to the tenets of the Declaration of Helsinki and was approved by the institutional review board of Jiangxi Provincial Maternal and Child Health Hospital. Clinical characteristics of the patients are summarized in Tables I-III.

DNA isolation. Tissue and peripheral blood DNA was isolated with E.Z.N.A. tissue (cat. no. D3396-02) and blood DNA kits (cat. no. D3392-02; Omega Bio-Tek, Inc., Norcross, GA, USA), respectively. The quantity of isolated DNA was assessed using a SmartSpec Plus spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

MED12 and MED12L mutational analysis. For the mutational analysis of MED12 (NM_005120.2) and the paralogous MED12L (NM_053002.5; Fig. 1), two pairs of primers were designed to amplify MED12 exons 1 and 2, and MED12L exon 2. The MED12 forward (5'-TTCTCCTGCCCTACTCTCCC-3') and reverse (5'-GGACCTGGATGGACATTG



Figure 1. Protein sequence homology analysis of MED12 and MED12L. MED12, mediator complex subunit 12; MED12L, mediator complex subunit 12 like.

CA-3') primers generated a 721 bp polymerase chain reaction (PCR) product; while the MED12L forward (5'-TGA AGCTTTTACATCCTTCTGCT-3') and reverse (5'-GGG CAGGACGGTATACATGG-3') primers generated a 296 bp PCR product. For each sample, 50 ng genomic DNA and 2 U LA Taq DNA polymerase (Takara Biotechnology Co., Ltd., Dalian, China) underwent 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 20 sec, in a total volume of 25 μ l. These reactions were performed in a Thermal Cycler 2720 (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The PCR products were subsequently sequenced on an ABI Prism 3730 DNA sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc.). Sequencing data was analyzed using DNASTAR Lasergene version 7.2 software (DNASTAR, Inc., Madison, WI, USA). The identified mutations were confirmed by bidirectional sequencing, and the somatic mutations of MED12 were verified by sequencing the paired peripheral blood lymphocytes. In addition, the protein sequences of human MED12 (NP_005111.2) and MED12L (NP_443728.3) were obtained from GenBank (24) to analyze the sequence homology.

Statistical analysis. Clinical data was taken at the time of sampling for the screening of molecular alterations. As the majority of patients with multiple leiomyomas harbored wild type and mutant MED12 in different tumors, the samples with multiple leiomyomas were excluded and the available clinical data from 135 patients with single intra-mural leiomyoma was obtained for statistical analysis. The serum levels of estradiol, progesterone, thyroid stimulating hormone, free triiodothyronine, free thyroxine, α -fetoprotein, carcinoembryonic antigen, squamous cell carcinoma antigen and cancer antigen 125 (CA125) were determined on day 3 of the menstrual cycle using a radioimmunoassay method, using their respective detection kits: estradiol (cat. no. 01010306024), progesterone (cat. no. 01010305024), thyroid stimulating hormone (cat. no. 01010201022), free triiodothyronine (cat. no. 01010202024), free thyroxine (cat. no. 01010203024), α -fetoprotein (cat. no. 01010102022), carcinoembryonic antigen (cat. no. 01010101022), and CA125 (cat. no. 01010104022) from Chemclin (Chemclin, Biotechnology Co., Ltd., Beijing, China), and squamous cell carcinoma antigen (cat. no. 68111LP15) was from Abbott Diagnostics (Santa Clara, CA, USA), as described previously (25-27). Statistical analyses were performed with SPSS software, version 17.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables were expressed as the mean \pm standard deviation

Table I. Association of mediator complex subunit 12 mutations with clinical characteristics in 135 uterine leiomyomas.

Features	Sample, n	Wild type, n	Wild type	Mutant type, n	Mutant type	P-value
Total	135	86		49		
Age (years)	135	86	41.51±6.52	49	39.61±7.09	0.12
Duration of disease (months)	135	86	30.05±41.65	49	32.19±39.46	0.78
Age of menarche (years)	129	82	14.61±1.64	47	14.11±1.46	0.088
Age of the first delivery (years)	117	76	23.07±2.62	41	22.88±2.63	0.718
Number of pregnancies (times)	135	86	3.05±1.49	49	3.06±1.64	0.96
Number of deliveries (times)	135	86	1.67±0.89	49	1.63±0.95	0.85
Number of abortions (times)	135	86	1.37±1.49	49	1.39±1.27	0.95
Cervical diameter (cm)	132	84	3.15±0.32	48	3.04±0.22	0.030
Haemoglobin (g/l)	135	86	109.09±21.60	49	111.53±21.10	0.53
Estrogen level (pg/ml)	74	44	157.02±137.44	30	135.89±111.65	0.49
Progesterone (ng/ml)	66	39	1.48±3.31	27	0.76±0.86	0.27
TSH (mIU/ml)	128	83	2.99±2.04	45	3.19±4.43	0.73
FT3 (pg/ml)	128	83	2.89±0.35	45	2.86±0.38	0.70
FT4 (ng/dl)	128	83	1.20±0.19	45	1.20±0.13	0.84
AFP (ng/ml)	130	83	2.28±1.43	47	2.00±0.79	0.23
CEA (ng/ml)	130	83	1.21±0.631	47	1.18±0.64	0.79
CA125 (μ/ml)	131	84	17.97±11.06	47	27.21±58.83	0.16
SCC (ng/ml)	129	82	1.74±1.52	47	1.65±1.25	0.73
HPV infection (pg/ml)	92	61	3.61±21.38	31	4.14±13.73	0.90
White blood cell count (x10 ⁹)	135	86	5.95±2.02	49	5.64±1.65	0.37
Lymphocyte cell count (x10 ⁹)	135	86	1.73±0.51	49	1.70±0.41	0.75
Mononuclear cell count (x10 ⁹)	135	86	0.48±0.18	49	0.43±0.14	0.15
Neutrophil count (x10 ⁹)	135	86	0.10±0.07	49	0.17±0.21	0.0023

AFP, α-fetoprotein; TSH, thyroid stimulating hormone; FT3, free triiodothyronine; FT4, free thyroxine; CEA, carcinoembryonic antigen; CA125, cancer antigen 125; SCC, squamous cell carcinoma; HPV, human papillomavirus.

Table II. Association between mediator complex subunit 12 mutations and clinical characteristics in 135 uterine leiomyomas.

Features	Wild type (n=86)		Mutant type (n=49)		P-value
	Regular, n	Irregular, n	Regular, n	Irregular, n	
Menstruation	79	7	48	1	0.26
Menstrual cycle	78	8	45	4	1.00
Caesarean birth					0.32
Yes	9		8		
No		77		41	
Cervical erosion	48	38	31	18	0.93

and analyzed by independent sample t-tests, and categorical variables were examined with Pearson's χ^2 test for the potential associations between MED12 mutations and the available biological variables. Clinical characteristics of interest were analyzed by logistic regression analysis to confirm statistical significance. All statistical tests were two-sided at the 5% level of significance. $P < 0.05$ was considered to indicate a statistically significant difference, and all P-values were two-tailed.

Results

Patient characteristics. Patient characteristics are presented in Tables I-III.

Mutation analysis of MED12 and MED12L in ULs. A total of 362 cases with ULs were analyzed for the presence of MED12 and MED12L mutations. MED12 mutations were identified in 158 out of 362 tumors (43.6%). There were 10 types of

Table III. Association between mediator complex subunit 12 mutations and clinical characteristics in 135 uterine leiomyomas.

Feature	Wild type, n			Mutant type, n			P-value
	Less	Median	More	Less	Median	More	
Menstrual blood							0.51
None	5			1			
Less		65			40		
Common			15			1	
Dysmenorrhea	72	6	8	35	12	16	0.24

Table IV. Mutation frequencies and forms of mediator complex subunit 12 in tumors with uterine leiomyomas.

Disease	Nucleotide change	Protein change	No. mutated samples, frequency (%)
Solitary			
	c.107G>T	p.L36R	5/290 (1.7)
	c.122T>A	p.V41E	1/290 (0.3)
	c.128A>C	p.Q43P	1/290 (0.3)
	c.130G>A	p.G44S	14/290 (4.8)
	c.130G>T	p.G44C	9/290 (3.1)
	c.130G>C	p.G44R	5/290 (1.7)
	c.131G>A	p.G44D	53/290 (18.3)
	c.131G>T	p.G44V	22/290 (7.6)
	c.131G>C	p.G44A	8/290 (2.8)
	c.133_150del 18	p.F45_A50del	3/290 (0.7)
Total multiple			121/290 (42.4)
	c.107G>T	p.L36R	4/72 (5.6)
	c.130G>A	p.G44V	2/72 (2.8)
	c.130G>T	p.G44C	4/72 (5.6)
	c.130G>C	p.G44R	4/72 (5.6)
	c.131G>A	p.G44D	14/72 (19.4)
	c.131G>T	p.G44S	2/72 (2.8)
	c.131G>C	p.G44A	7/72 (9.7)
Total combined			37/72 (51.4)
	c.107G>T	p.L36R	9/362 (2.5)
	c.122T>A	p.V41E	1/362 (0.3)
	c.128A>C	p.Q43P	1/362 (0.3)
	c.130G>A	p.G44S	16/362 (4.4)
	c.130G>T	p.G44C	13/362 (3.6)
	c.130G>C	p.G44R	9/362 (2.5)
	c.131G>A	p.G44D	67/362 (18.5)
	c.131G>T	p.G44V	24/362 (6.6)
	c.131G>C	p.G44A	15/362 (4.1)
	c.133_150del 18	p.F45_A50del	3/362 (0.8)
Total			158/362 (43.6)

mutation, including p.L36R (c.107T>G), p.V41E (c.122T>A), p.Q43P (c.128A>C), p.G44S (c.130G>A), p.G44C (c.130G>T), p.G44R (c.130G>C), p.G44D (c.131G>A), p.G44 V (c.131G>T), p.G44A (c.131G>C) and p.F45_A50del (c.133_150del 18; Fig. 2; Table IV). Of these mutations, the most prevalent

mutation was located on the 44th codon, and MED12 p.G44D (c.131G>A) was the most common mutation form (42.4%, 67/158). All patients with the mutation were heterozygous and retained a wild type allele, and the somatic status of this sample was confirmed by sequencing the paired peripheral

Table V. MED12 mutation analysis in concurrent tumors from patients with multiple leiomyomas.

Sample no.	Tumor no.	MED12 status
198	198T1	WT
198	198T2	c.107T>G, p.L36R
200	200T1	c.131G>A, p.G44D
200	200T2	WT
224	224T1	c.131G>T, p.G44V
224	224T2	c.130G>C, p.G44R
366	366T1	c.130G>C, p.G44R
366	366T2	c.131G>A, p.G44D
388	388T1	c.131G>A, p.G44D
388	388T2	c.107T>G, p.L36R
391	391T1	WT
391	391T2	WT
391	391T3	WT
411	411T1	WT
411	411T2	WT
411	411T3	WT
415	415T1	c.130G>T, p.G44C
415	415T2	c.130G>C, p.G44R
415	415T3	WT
416	416T1	c.131G>A, p.G44D
416	416T2	c.131G>T, p.G44V
420	420T1	c.131G>A, p.G44D
420	420T2	c.131G>A, p.G44D
420	420T3	c.131G>A, p.G44D
422	422T1	c.130G>T, p.G44C
422	422T2	c.131G>C, p.G44A
423	423T1	c.130G>T, p.G44C
423	423T2	WT
424	424T1	c.130G>T, p.G44C
424	424T2	c.131G>C, p.G44A
459	459T1	WT
459	459T2	c.131G>C, p.G44A
481	481T1	c.107T>G, p.L36R
481	481T2	WT
481	481T3	WT
482	482T1	WT
482	482T2	WT
488	488T1	c.130G>A, p.G44S
488	488T2	WT
489	489T1	WT
489	489T2	WT
491	491T1	c.131G>A, p.G44D
491	491T2	c.131G>C, p.G44A
494	494T1	c.131G>C, p.G44A
494	494T2	c.130G>A, p.G44S
497	497T1	WT
497	497T2	WT
498	498T1	WT
498	498T2	WT
499	499T1	c.131G>A, p.G44D
499	499T2	WT

Table V. Continued.

Sample no.	Tumor no.	MED12 status
500	500T1	c.131G>A, p.G44D
500	500T2	c.131G>A, p.G44D
501	501T1	WT
501	501T2	WT
504	504T1	WT
504	504T2	WT
506	506T1	WT
506	506T2	c.107T>G, p.L36R
524	524T1	WT
524	524T2	WT
530	530T1	c.131G>A, p.G44D
530	530T2	c.131G>A, p.G44D
530	530T3	c.131G>C, p.G44A
531	531T1	c.131G>C, p.G44A
531	531T2	c.131G>A, p.G44D
531	531T3	WT
576	576T1	WT
576	576T2	WT
580	580T1	c.130G>C, p.G44R
580	580T2	WT
580	580T3	WT

WT, wild type; MED12, mediator complex subunit 12.

blood (Fig. 2). In contrast to the frequent MED12 mutations, no MED12L mutations were identified in the 362 samples with ULs (Fig. 2).

MED12 mutations in multiple ULs. To determine the frequency of clonally associated ULs in the samples, 72 concurrent tumors from 32 patients with multiple leiomyomas were analyzed for the presence of MED12 mutations. A total of 45 tumors from 20 patients exhibited discordant mutation status of MED12, indicating that these tumors were clonally independent (Table V).

MED12 mutations in adjacent control myometrium. A total of 145 adjacent control myometrium adjacent to MED12-mutated tumors were analyzed for the presence of MED12 mutations by direct DNA sequencing. Two myometrium (2/145, 1.4%) were revealed to harbor MED12 mutations. Control myometrium from samples 387 and 395 harbored MED12 p.G44D (c.131G>A) and p.G44S (c.130G>A) mutations, respectively (Table VI), while the remaining 143 myometrium did not harbor MED12 mutations.

Association between MED12 mutations, clinical features and biological characteristics. Of the 322 patients, MED12-mutated tumors tended to have smaller cervical diameters [odds ratio (OR)=0.181; 95% confidence interval, 0.036-0.919; P<0.05; Tables I and VII]. However, MED12 mutations were not associated with any other clinical characteristics, including age of menarche and the first delivery, number of pregnancies,

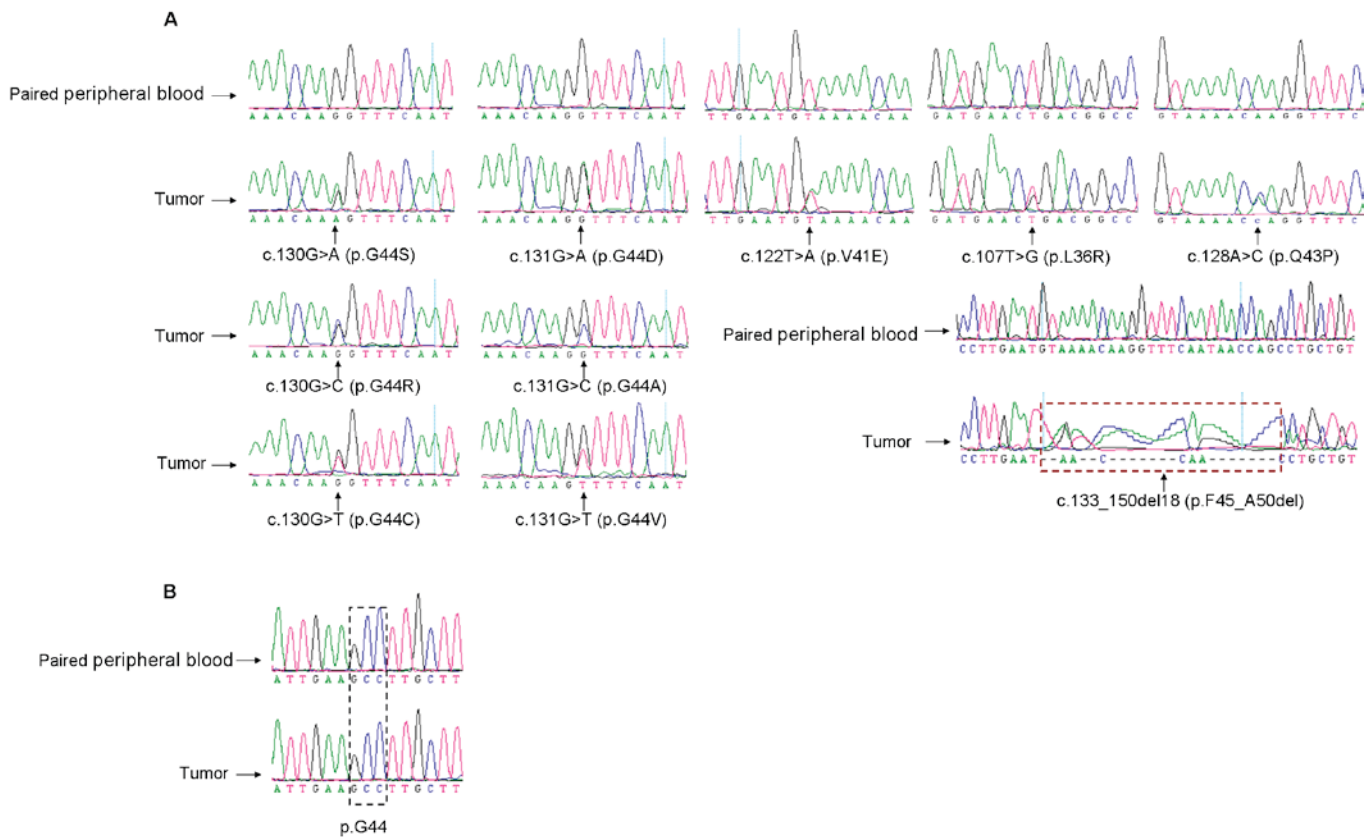


Figure 2. Representative sequencing electropherograms of (A) mediator complex subunit 12 and (B) mediator complex subunit 12-like. Arrows refer to the locations of the mutation.

Table VI. MED12 mutation analysis in adjacent control myometrium in patients with uterine leiomyomas.

Patient number	Tissue	MED12 mutation
387	Leiomyoma	c.130G>A, p.G44S
	Adjacent myometrium	c.131G>A, p.G44D
395	Leiomyoma	c.130G>C, p.G44R
	Adjacent myometrium	c.130G>A, p.G44S

MED12, mediator complex subunit 12.

deliveries and abortions, menstrual cycle and menstruation, or serum levels of estradiol, progesterone and CA125 (Tables I-III).

Discussion

Prevalent MED12 mutations have been detected in ULs (8-12), breast fibroadenoma (13,14) and chronic lymphocytic leukemia (15). Evolutionary conservation analysis results have demonstrated that these mutations were evolutionarily conserved from yeast to humans (18). Furthermore, transgenic mice expressing MED12 p.G44D mutations demonstrated increased genomic instability, uterine hyperplasia and ULs (20).

Previous studies have revealed variable frequencies of MED12 mutations in ULs, ranging from 0-84.1%

Table VII. Logistic regression analysis demonstrating that mediator complex subunit 12 mutations are associated with smaller cervical diameters in uterine leiomyomas.

Feature	Odds ratio (95% confidence interval)	P-value
Cervical diameter	0.181 (0.036-0.919)	0.039

(7,8,10-12,28-37). Among which, MED12 mutation frequencies in Han Chinese UL samples were 29.3% (49/167) (35), 52.5% (95/181) (33) and 54.4% (93/171) (38), respectively, within the previously observed frequency. In the present study, the mutation frequency of MED12 in the UL samples was 158/362 (43.6%), within the range of these previous observations (7,8,10-12,28-37). In addition, the prevalent MED12 mutations in Han Chinese ULs observed in the present study as well as previous studies (35,38) indicated that MED12 mutations may be actively involved in the pathogenesis of ULs in Han Chinese patients. It was hypothesized that the variation of MED12 mutations in ULs may be caused by disease heterogeneity, as ULs are subdivided into multiple different subtypes (28,35,37).

MED12 mutations have been investigated broadly in samples with ULs (7,8,10-12,28-37). However, only one study has explored the potential associations between MED12 and several clinical features, which analyzed a total of 164 ULs

from Finnish patients and identified that MED12 mutations were associated with smaller tumor size and a larger number of tumors in multiple leiomyomas (12). Similarly, the present results also revealed that patients with MED12-mutated tumors tended to have smaller cervical diameters when compared with patients with MED12 wild type tumors. The consistent observations in this previous and present study indicated that MED12 mutations were inversely associated with cervix size in ULs. In addition, in the present study, no associations were observed between MED12 mutations and other available clinical data, including age of menarche and the first delivery, numbers of pregnancies, deliveries and abortions, menstrual cycle and menstruation, or serum levels of estradiol, progesterone and CA125 (12).

Previous studies have also attempted to determine whether MED12 mutations also existed in the adjacent control myometrium of ULs (7,31,34,39,40). MED12 mutations were identified in 2 out of 13 (15%) cases in myometrium adjacent to leiomyomas (39), while several other studies did not detect MED12 mutations in the control myometrium (7,31,34,40). A low frequency of MED12 mutations was detected in 2 out of 145 (1.4%) adjacent control myometrium with MED12 mutations in the tumors (Table IV). The exact reason for the differential MED12 mutation frequency in control myometrium was unclear, however, the presence of MED12 mutations in the adjacent myometrium in ULs indicated that MED12 mutation may be an early event in the development of ULs in certain cases.

The MED12 mutation status in concurrent tumors from multiple leiomyomas supported several previous observations that the majority of these tumors arise independently (7,9).

In contrast to the high frequency of MED12 mutations in ULs, no mutations were detected in the paralogous MED12L in the present sample cohort, indicating that MED12L mutations may not be common in ULs.

In summary, the present study observed prevalent MED12 mutations in UL samples. Positive MED12 mutations were associated with the diameter of uterine cervix in ULs. Furthermore, MED12 mutations were observed at low frequencies in adjacent control myometrium in ULs, indicating that MED12 mutation may be an early event in the pathogenesis of a proportion of ULs. Furthermore, MED12 mutation status in concurrent tumors from multiple leiomyomas supported several prior observations that the majority of these tumors arise independently.

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