DOCTORAL THESIS

MED12 mutations in uterine leiomyomas: prediction of volume reduction by gonadotropin-releasing hormone agonists

（*MED12* 遺伝子変異解析に基づく子宮筋腫の縮小予測）

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GYNECOLOGY

MED12 mutations in uterine leiomyomas: prediction of volume reduction by gonadotropin-releasing hormone agonists

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BACKGROUND: Gonadotropin-releasing hormone agonists are used to treat premenopausal uterine leiomyomas; however, leiomyoma volume reduction is not always achieved. The reduction rate after this treatment varies for each leiomyoma, even in the same patient. Therefore, an effective method for predicting uterine leiomyoma volume reduction is required to reduce the adverse hypoestrogenic effects and drug-related economic burden related to gonadotropin-releasing hormone agonists.

OBJECTIVE: This study aimed to determine the predictive use of *MED12* mutations for evaluating the effect of gonadotropin-releasing hormone agonist treatment concerning reducing uterine leiomyoma volume and to predict the *MED12* mutation status based on the findings of magnetic resonance imaging performed before treatment.

STUDY DESIGN: *MED12* exon 2 mutation and erythropoietin expression in uterine leiomyomas were evaluated concerning volume reduction, as measured using magnetic resonance imaging. We developed a system for classifying leiomyomas according to T2-weighted magnetic resonance imaging signals to noninvasively predict the presence or absence of *MED12* mutations in leiomyomas. Leiomyoma samples (>5 cm) were obtained from 168 patients during surgery (hysterectomy or myomectomy) between 2005 and 2021 at Yokohama City University Hospital. To analyze the rate of leiomyoma volume reduction, 41 patients had been preoperatively administered the gonadotropin-releasing hormone agonist (leuprorelin acetate 3.75 mg, monthly subcutaneous injection) for 3 months; magnetic resonance imaging was performed before and after treatment without contrast material.

RESULTS: Patients with *MED12* exon 2 mutations had smaller volume reduction after treatment with the gonadotropin-releasing hormone agonist ($P<.001$, Mann-Whitney *U* test) and displayed lower signal intensity on T2-weighted images than those with leiomyomas expressing wild-type *MED12* exon 2. The newly proposed magnetic resonance imaging–based classification system showed that *MED12* exon 2 mutations were more frequent in the low-signal group than in the high-signal group, with nearly equal proportions of mutated and wild-type *MED12* exon 2 leiomyomas noted in the intermediate group. The low-signal group had significantly lower erythropoietin expression levels than the high-signal group ($P<.001$, Kruskal-Wallis test with the Dunn posthoc analysis).

CONCLUSION: *MED12* mutation status can be a candidate marker for predicting the effect of gonadotropin-releasing hormone agonists on uterine leiomyoma reduction. Magnetic resonance imaging findings can be used to determine *MED12* mutation status as a noninvasive strategy to select patients who will most likely benefit from gonadotropin-releasing hormone agonist treatment.

Key words: erythropoietin, genomics, gonadotropin-releasing hormone analogue, mediator complex subunit 12, magnetic resonance imaging classification, mutation status, predictive marker, uterine fibroid, vessel maturity, volume reduction rate

Introduction

During treatment of premenopausal uterine leiomyomas, gonadotropin-releasing hormone (GnRH) agonists and antagonists are commonly used to decrease female hormone levels via their eventual inhibitory effect on gonadotropin secretion to alleviate pain symptoms and suppress hypermenorrhea.[1–3] Preoperative GnRH agonist use can reduce uterine volume and alleviate anemia, which, in turn, can reduce blood loss,[4] operation time, and complication rates during surgery.[5] However, ideal reduction of uterine leiomyoma volume is not always achieved with GnRH agonists.[6,7]

The degree of reduction of uterine leiomyoma volume after such treatment varies for each leiomyoma, even in the same patient. Therefore, a method for effectively predicting volume reduction is required.[7–12] A predictive method based on signal enhancement rate measured through contrast-enhanced magnetic resonance imaging (MRI) had been proposed.[10–12] However, this method is not widely used to predict the effect of GnRH agonists because clinicians do not routinely use enhancement MRI for leiomyomas predicted to be benign.

The mechanisms underlying the clinical differences observed in response to GnRH agonists remain unclear. Failure of GnRH agonist treatment to reduce uterine leiomyoma volume delays surgery. Moreover, this pseudomenopause treatment is associated with adverse drug reactions, such as climacteric symptoms and decreased bone density,[13–17] thereby contributing to the drug-related economic burden. If the reduction effect can be predicted before treatment, clinicians can select patients most likely to benefit from it, thereby simultaneously decreasing the medical economic burden.[18–22]

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AJOG at a Glance

Why was this study conducted?

The predictive use of mediator complex subunit 12 (*MED12*) mutations (MUTs) for evaluating the effectiveness of gonadotropin-releasing hormone (GnRH) agonists for leiomyoma volume reduction needed to be determined. A magnetic resonance imaging (MRI)-based classification system for predicting *MED12* MUTs, erythropoietin (EPO) expression, and leiomyoma volume reduction rate would help reduce adverse hypoestrogenic effects induced by GnRH agonists and economic burden.

Key findings

The leiomyoma volume reduction rate by GnRH agonist treatment of *MED12* wild-type leiomyomas was significantly higher than that of leiomyomas with *MED12* exon 2 MUT. The classification of leiomyomas according to signal intensity on T2-weighted MRI was useful in estimating the presence or absence of *MED12* exon 2 MUTs.

What does this add to what is known?

MED12 MUT status has been found to be a candidate predictive marker for the therapeutic effect of GnRH agonists. Non–contrast-enhanced MRI findings obtained before GnRH agonist therapy can reflect *MED12* MUT status and EPO expression in leiomyomas.

The mechanisms underlying uterine leiomyoma development and growth remain unclear. Uterine leiomyomas frequently show somatic site-specific mutations (MUTs) in exon 2 of the mediator complex subunit 12 (*MED12*) gene,[23–27] whose roles in leiomyoma development, growth, and reduction are not sufficiently understood, despite being the most common MUTs noted in leiomyoma. In our previous study,[28,29] we found that leiomyomas with high erythropoietin (EPO) expression were larger and had more mature vessels than leiomyomas with low EPO expression, which suggested that EPO expression in leiomyomas is advantageous for tumor growth because of the vessel-maturing effect. We found that *MED12* wild-type (WT) uterine leiomyomas had higher EPO messenger RNA (mRNA) expression levels than uterine leiomyomas with *MED12* MUTs.[29] Moreover, only *MED12* WT leiomyoma cells showed elevated EPO mRNA expression in response to estrogen exposure in vitro.[29]

Given that *MED12* WT leiomyomas express EPO in an estrogen-dependent manner and are larger than leiomyomas with *MED12* MUTs, we hypothesized that the size of *MED12* WT leiomyomas is more likely to reduce in response to estrogen decrease via GnRH agonist treatment. Therefore, in this study, we aimed to determine (1) whether the *MED12* MUT status is associated with leiomyoma volume reduction induced by GnRH agonists, (2) whether it is possible to predict *MED12* MUT status based on the findings of MRI performed before GnRH agonist treatment, and (3) and whether the EPO expression level can be predicted on the basis of MRI findings.

Materials and Methods

Patients and tissue samples

This study was approved by the Ethics Committee of Yokohama City University Graduate School of Medicine (institutional review board approval number A150 122017; July 27, 2012). The study was following the ethical standards for human experimentation established in the Declaration of Helsinki. Written informed consent was obtained from all the study participants. The study included 168 uterine leiomyoma tissue samples obtained from women who had undergone hysterectomy or myomectomy during 2005 to 2021 at Yokohama City University Hospital after the diagnosis of uterine leiomyoma via physical examination. Some samples used in our previous studies were also included in the current study.[28,29]

To analyze the rate of leiomyoma reduction because of GnRH agonist treatment, 41 patients who had been preoperatively treated with a GnRH agonist (leuprorelin) and for whom data were available on leiomyoma volume reduction were included. The following types of patients were excluded: patients who did not undergo MRI before or after GnRH agonist treatment, those who were treated with a GnRH agonist other than leuprorelin (ie, intranasal buserelin and nafarelin, because adherence to drug therapy was not assessed in these patients. There was no patient treated with other injectable GnRH agonists.), and those who had undergone GnRH agonist treatment before enrollment. In addition, patients with a leiomyoma whose diameter was $\leq$5 cm before GnRH agonist treatment was initiated were excluded.

Messenger RNA expression and *MED12* mutation analyses

The specimens were obtained during surgery, snap-frozen in liquid nitrogen, and stored at −80°C until RNA and DNA analyses were performed. Real-time reverse transcription–polymerase chain reaction for EPO and beta-actin (ACTB) mRNA levels and Sanger sequencing of *MED12* exon 2 MUT hotspots were performed using methods described in our previous studies.[28,29] EPO protein level was not measured in this study because we have already shown that EPO protein is produced concomitant with EPO mRNA expression with a high correlation in our previous study.[28] We defined the leiomyomas with *MED12* exon 2 MUT as *MED12* MUT leiomyomas and the leiomyomas with WT *MED12* exon 2 as *MED12* WT leiomyomas. This study did not assess the MUTs in *MED12* exon 1, found in <1% of the leiomyomas.[30]

Magnetic resonance imaging–based measurement of leiomyoma reduction rate

All the patients underwent MRI without contrast agent before and after GnRH

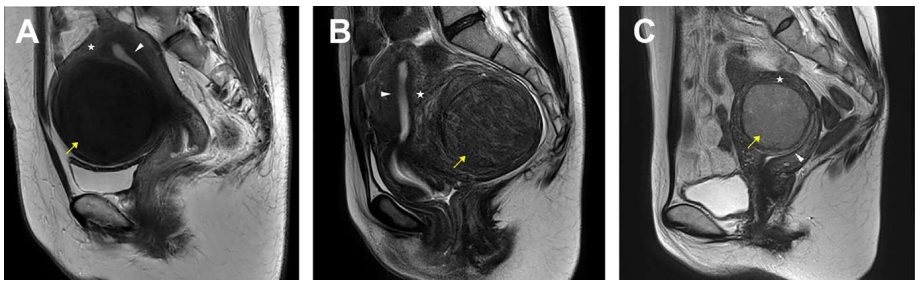
agonist (leuprorelin) treatment to assess changes in leiomyoma size. MRI was performed using a 1.5-T system (Symphony; Siemens, Erlangen, Germany) with a body matrix phased array coil (n=41). All 41 patients included in the study were treated with leuprorelin acetate (3.75 mg, monthly subcutaneous injection) for 3 months. The nodule size was again measured using MRI at 13 to 14 weeks after the first leuprorelin dose. The size of the largest leiomyoma was measured for patients with multiple leiomyomas. The leiomyoma volume was calculated by applying the equation for ellipsoid volume (length × width × depth × $\pi/6$) to T2-weighted images (considering the maximum length of each coordinate) and has been expressed in terms of fold change compared with the pretreatment volume.[7,9–12] The reduction rate was calculated as the ratio of leiomyoma volume before and after treatment for the largest leiomyoma.

New magnetic resonance imaging–based classification

To examine the association between the presence or absence of *MED12* MUT and non–contrast-enhanced MRI findings before GnRH agonist treatment, we developed a new classification system based on leiomyoma signal intensity obtained using T2-weighted imaging. A total of 168 patients were included in this analysis.

The samples were classified according to the signal intensity noted in T2-weighted images for the major part (≥50%) of each leiomyoma (Figure 1): low group, leiomyomas with signal intensity similar to or lower than that of the junctional zone (Figure 1, A); intermediate group, leiomyomas whose signal intensity was intermediate, that is, higher than that of the junctional zone and lower than that of the surrounding normal muscle layers (Figure 1, B); and high group, leiomyomas with signal intensity similar to or higher than that of the surrounding normal muscle layers (Figure 1, C). Leiomyomas, wherein the major part accounted for <50% because of the mixing of various signals, were excluded from the analysis.

FIGURE 1
MRI-based classification of leiomyomas for *MED12* mutation identification



Non–contrast-enhanced T2-weighted MRI of uterine leiomyomas for classification into 3 groups before GnRH agonist treatment. **A,** Low group, characterized by nodules with signal intensity similar to or lower than that of the junctional zone. **B,** Intermediate group, characterized by nodules with signal intensity lower than that of the myometrium and higher than that of the junctional zone. **C,** High group, characterized by nodules with signal intensity similar to or higher than that of the myometrium. *Yellow arrows* indicate the nodules in each panel, *white arrowheads* indicate the junctional zone, and *stars* indicate the myometrium.

GnRH, gonadotropin-releasing hormone; *MED12*, mediator complex subunit 12; *MRI*, magnetic resonance imaging.

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Vessel maturity and density

The vessel maturity and density of the leiomyoma tissue were evaluated histologically. The vessels were classified into the low and high maturity groups using our previously described measurement and scoring method involving hematoxylin and eosin staining and CD34 immunohistochemistry.[28] The associations between the presence or absence of *MED12* MUTs and vessel maturity and density were examined. Samples from 54 patients were included in this analysis; of these, 25 patients had been treated with the GnRH agonist.

Statistical analysis

All statistical analyses were performed using the IBM SPSS Statistics for Windows (version 28; IBM Corporation, Armonk, NY). The uterine leiomyoma volume reduction rate and gene expression levels were compared using the Mann-Whitney *U* test and the Kruskal-Wallis test, followed by the Dunn post-hoc analysis with the Bonferroni correction. The odds ratio (OR) for the associations was calculated using logistic regression, along with the 95% confidence interval (CI) and *P* value. The Fisher exact test was used to analyze the relationship between *MED12* MUTs and tumor vessel maturity. Statistical significance was set at $P<.05$.

Results

Association between *MED12* mutations and volume reduction rate

Table 1 shows the characteristics of the 41 patients for whom the leiomyoma reduction rate was calculated using MRI before and after GnRH agonist treatment. All 41 patients (mean age, 42.7 years) were premenopausal. *MED12* MUTs were detected in 21 of 41 samples (51%). The background characteristics for samples with *MED12* MUTs did not significantly differ from those for samples without *MED12* MUTs in terms of age, body mass index (BMI), nulligravida, nulliparity, hemoglobin levels, or maximum leiomyoma diameter.

Figure 2 shows the volume reduction rate for the 20 patients with *MED12* WT and 21 patients with *MED12* MUT leiomyomas. The leiomyoma volume reduction rate of patients with *MED12* WT leiomyomas (median, 43.4% reduction) was significantly higher than that of patients with *MED12* MUT leiomyomas (median, 15.6% reduction) ($P<.001$, Mann-Whitney *U* test). Thus, the leuprorelin-induced volume

TABLE 1
Characteristics of the 41 participants who underwent GnRH agonist therapy in this study

Characteristics	*MED12* WT (n=20)	*MED12* MUT (n=21)	*P* value
Age (y), median (IQR)	45.0 (42.8–47.3)	43.0 (39.0–44.0)	.175
BMI (kg/m^2), median (IQR)	23.1 (21.5–25.1)	24.2 (21.8–25.8)	.492
Nulligravida, n (%)	10 (50.0)	15 (71.4)	.278
Nulliparity, n (%)	12 (60.0)	16 (76.2)	.437
Hemoglobin level (g/dL), median (IQR)	13.1 (12.5–13.7)	13.3 (12.0–13.8)	.908
Max leiomyoma length (cm), median (IQR)	8.5 (7.7–9.5)	9.0 (6.9–10.2)	.379

BMI, body mass index; *GnRH*, gonadotropin-releasing hormone; *IQR*, interquartile range; *MED12*, mediator complex subunit 12; *MUT*, mutation; *WT*, wild type.

Nagai. Magnetic resonance imaging–based classification of leiomyomas for MED12 mutation identification. Am J Obstet Gynecol 2023.

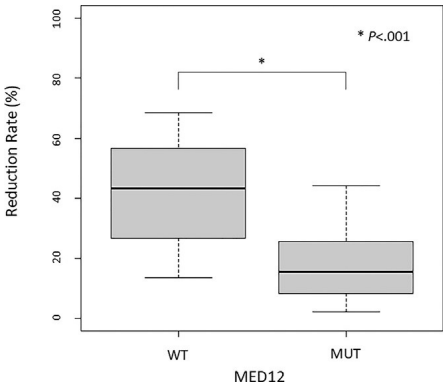
reduction was lower in *MED12* MUT leiomyomas than in *MED12* WT leiomyomas.

New magnetic resonance imaging–based classification and *MED12* mutations

The 168 leiomyoma samples were classified into the low, intermediate, or high groups based on MRI analyses before GnRH agonist treatment; of these, 82 samples were *MED12* MUT leiomyomas. The background characteristics of *MED12* WT and *MED12* MUT leiomyomas did not significantly differ in terms of age, BMI, nulligravida, nulliparity, menopause, GnRH agonist therapy, hemoglobin level, or maximum leiomyoma diameter (Table 2). The Supplemental Table summarizes the frequency and type of *MED12* MUTs identified.

Table 3 shows the association between the new MRI-based classification and *MED12* MUT frequency. The low group (n=68) included 56 patients with *MED12* MUT and 12 patients with *MED12* WT leiomyomas, representing a *MED12* MUT frequency of 82.4%. The intermediate group (n=44) consisted of 23 patients with *MED12* MUT and 21 patients with *MED12* WT leiomyomas, with a MUT frequency of 52.3%. The high group (n=56) consisted of 3 patients with *MED12* MUT and 53 patients with *MED12* WT leiomyomas, with a MUT frequency of 5.4%. The OR (95% CI) for predicting *MED12* MUTs in the low, intermediate, and high groups according to the new MRI-based classification was 13.28 (6.17–28.60), 1.21 (0.61–2.40), and 0.02 (0.01–0.08), respectively (Table 3). The *MED12* MUT frequency was higher in the low group, whereas the *MED12* WT frequency was higher in the high group.

FIGURE 2
Leiomyoma volume reduction induced by GnRH agonist and *MED12* mutation



The rate of uterine leiomyoma volume reduction in *MED12* WT leiomyomas was significantly higher than that in *MED12* MUT leiomyomas. The *P* value was calculated using the Mann-Whitney *U* test.

GnRH, gonadotropin-releasing hormone; *MED12*, mediator complex subunit 12; *MUT*, mutation; *WT*, wild type.

Nagai. Magnetic resonance imaging–based classification of leiomyomas for MED12 mutation identification. Am J Obstet Gynecol 2023.

In addition, the volume reduction rate increased from the low group (18 [15.1%]) to the intermediate group (13 [35.2%]) to the high group (10 [46.9%]) (Figure 3). The volume reduction rate in the low group was significantly lower than that in the high (*P*<.001) and intermediate (*P*=.002) groups, with no significant difference noted between the high and intermediate groups (*P*=.253).

New magnetic resonance imaging–based classification and erythropoietin expression

Figure 4 shows the associations of the proposed MRI-based classification with the relative EPO expression levels in uterine leiomyomas, normalized to the mRNA level of the reference ACTB. The relative EPO expression level in the low group was significantly lower than that in the high (*P*<.001) and intermediate (*P*=.018) groups, with no significant difference noted between the high and intermediate groups (*P*=.547).

MED12 mutations and vessel maturity

Immunohistochemistry findings showed that the number of samples with a high vessel maturity score was significantly higher for *MED12* WT than for *MED12* MUT leiomyomas (*P*<.001, the Fisher exact test) (Figure 5). However, vessel density did not significantly differ between the groups.

Comment

Principal findings

This study reported 3 novel findings that can help stratify and select patients who have uterine leiomyomas and would benefit from GnRH agonist treatment.

First, we identified an association between the leiomyoma volume reduction rate and *MED12* MUTs: *MED12* MUT leiomyomas were less likely to show a decrease in size than *MED12* WT leiomyomas after GnRH agonist treatment. Thus, the presence or absence of *MED12* MUTs can be a candidate marker for predicting the effect of GnRH agonists on uterine leiomyoma volume reduction, and a method for predicting the *MED12* MUT status would be useful.

TABLE 2
Characteristics of all the study participants

Characteristics	*MED12* WT (n=86)	*MED12* MUT (n=82)	*P* value
Age (y), median (IQR)	44.0 (40.0–48.0)	43.5 (39.0–48.0)	.194
BMI (kg/m^2), median (IQR)	22.5 (20.6–25.0)	24.1 (20.6–25.9)	.869
Nulligravida, n (%)	31 (36.0)	35 (42.7)	.470
Nulliparity, n (%)	38 (44.2)	42 (51.2)	.449
Menopause, n (%)	8 (9.3)	6 (7.3)	.852
GnRH agonist therapy, n (%)	32 (37.2)	31 (37.8)	>.999
Hemoglobin level (g/dL), median (IQR)	12.4 (11.3–13.1)	12.4 (11.6–13.4)	.705
Max leiomyoma diameter (cm), median (IQR)	8.8 (6.9–12.0)	8.5 (6.5–10.6)	.088

BMI, body mass index; *GnRH*, gonadotropin-releasing hormone; *IQR*, interquartile range; *MED12*, mediator complex subunit 12; *MUT*, mutation; *WT*, wild type.

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Second, we identified an association between the T2-weighted MRI findings and *MED12* MUT status, with *MED12* MUT and *MED12* WT leiomyomas tending to display low and high signal intensities, respectively. Considering these findings, we developed a new classification system for uterine leiomyomas based on T2-weighted MRI results obtained before GnRH agonist treatment (low, intermediate, and high groups), wherein the frequency of *MED12* MUTs was found to differ between the 3 groups significantly. These results indicated that non–contrast-enhanced MRI findings obtained before GnRH agonist treatment can be used to predict the presence or absence of *MED12* MUTs in leiomyomas.

Third, the analysis of the 3 groups according to the new MRI-based classification showed that the low group had significantly lower EPO expression than the intermediate and high groups. The results confirmed that the *MED12* MUT status is strongly correlated with EPO expression, as already shown in our previous study.[29]

Results in the context of what is known

Confirming the presence of *MED12* MUTs before initiating GnRH agonist treatment is highly informative for effective leiomyoma volume reduction. Needle biopsy of leiomyomas is a simple method that can be used to predict leiomyoma volume reduction by GnRH agonists.[31,32] Although the *MED12* MUT status could help predict the treatment response, leiomyoma specimens are needed for genetic analyses, and as needle biopsy is an invasive technique, it is not routinely performed in our practice. Therefore, we propose to use our new MRI-based classification for predicting *MED12* MUT status and volume reduction by GnRH agonists.

In addition, researchers have attempted to predict the effect of GnRH agonists on uterine leiomyomas by performing contrast-enhanced MRI with a gadolinium contrast agent before GnRH agonist treatment. The enhanced area was measured using the T1-weighted image showing the maximum leiomyoma diameter, and the enhancement ratio (percentage of the enhanced area to total leiomyoma area) was found to be strongly correlated with leiomyoma volume reduction.[11] However, contrast-enhanced MRI is not usually performed for benign uterine leiomyomas.

Our new method is a noninvasive, simple method for MRI-based classification of leiomyomas to predict the presence or absence of *MED12* MUTs and the effect of GnRH agonists. Using this method, leiomyomas are classified into 3 groups based on signal intensity relative to that of the junctional zone and normal muscle layer, using only T2-weighted MRI without contrast enhancement.

T2-weighted imaging is mainly based on tissue water content. Water has a more homogeneous microenvironment than soft tissues and exhibits slower decay of signal intensity over time (long

TABLE 3
Agreement between MRI-based classification of leiomyomas and *MED12* MUT frequency

Classification	*MED12* WT (n=86)	*MED12* MUT (n=82)	MUT frequency	OR	95% CI	*P* value
Low group (n=68)	12	56	82.4%	13.28	6.17–28.60	.031
Intermediate group (n=44)	21	23	52.3%	1.21	0.61–2.40	.590
High group (n=56)	53	3	5.4%	0.02	0.01–0.08	<.001

The low group is defined as leiomyomas with signal intensity similar to or lower than that of the junctional zone; the intermediate group is defined as leiomyomas with signal intensity lower than that of the myometrium and higher than that of the junctional zone; and the high group is defined as leiomyomas with signal intensity similar to or higher than that of the myometrium.

CI, confidence interval; *MED12*, mediator complex subunit 12; *MRI*, magnetic resonance imaging; *MUT*, mutation; *OR*, odds ratio; *WT*, wild type.

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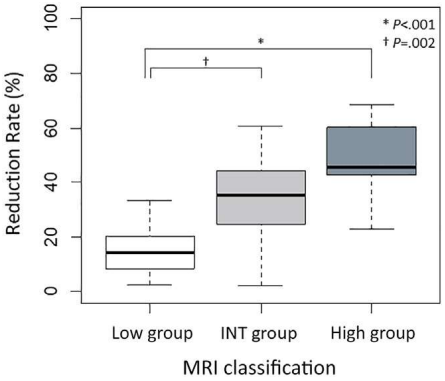
FIGURE 3
Association between leiomyoma volume reduction rate and MRI-based classification



The volume reduction rate increased from the low to the intermediate to the high groups. The leiomyomas in the intermediate group included those with characteristics of the leiomyomas in the low and high groups because of the almost equal proportions of *MED12* WT and *MED12* MUT leiomyomas in the intermediate group. Low, intermediate, and high groups were based on the proposed classification according to MRI signals. The *P* value was calculated using the Kruskal-Wallis test followed by the Dunn post-hoc analysis with the Bonferroni correction.

INT, intermediate; *MED12*, mediator complex subunit 12; *MRI*, magnetic resonance imaging; *MUT*, mutation; *WT*, wild type.

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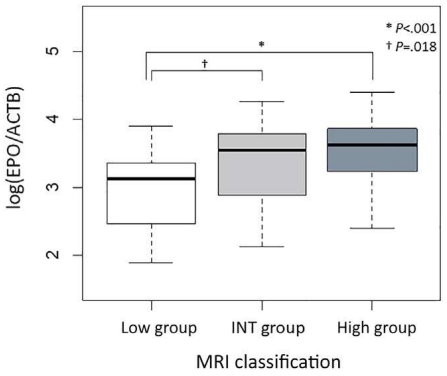
FIGURE 4
Association between EPO mRNA expression level in leiomyomas and MRI-based classification



The relative EPO expression level in the low group was significantly lower than that in the high and intermediate groups. Low, intermediate, and high groups were based on the proposed classification according to MRI signals. The *P* value was calculated using the Kruskal-Wallis test followed by the Dunn posthoc analysis with the Bonferroni correction.

ACTB, beta-actin; *EPO*, erythropoietin; *INT*, intermediate; *mRNA*, messenger RNA; *MRI*, magnetic resonance imaging.

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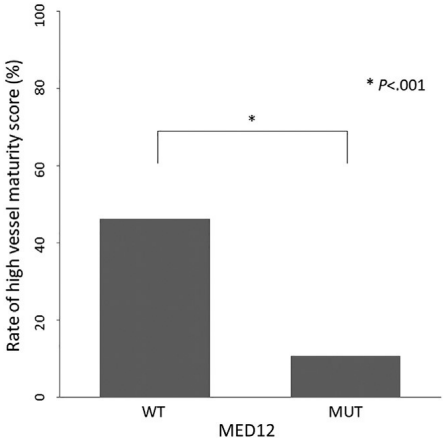
FIGURE 5
Association between leiomyoma vessel maturity and *MED12* mutation



Comparison of uterine leiomyoma vessel maturity in *MED12* WT (n=26) and *MED12* MUT (n=28) leiomyomas using the scoring system described in our previous study[28] to evaluate each leiomyoma. The number of leiomyomas with a high vessel maturity score (4 or 5) was significantly higher for *MED12* WT leiomyomas than for *MED12* MUT leiomyomas ($P<.001$). The *P* value was calculated using the Fisher exact test.

MED12, mediator complex subunit 12; *MUT*, mutation; *WT*, wild type.

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T2 relaxation time).[33] Thus, water appears hyperintense relative to soft tissue structures on T2-weighted images.[33] The presence or absence of the *MED12* MUT may be associated with the water content of the leiomyoma tissue. *MED12* WT leiomyomas show higher EPO expression,[29] which increases vessel maturation and may lead to increased water content in the tissue, than leiomyomas with the *MED12* exon 2 MUT. To examine this association, the leiomyoma water content needs to be measured, for example, by comparing fresh and dry tumor weights.

Clinical implications

Uterine leiomyoma is an important focus area in the obstetrics and gynecology fields, with extensive efforts being focused on providing personalized therapy. Various methods, including endoscopic surgery and medical treatment options (eg, GnRH agonists, GnRH antagonists, and ulipristal acetate), have been used to treat uterine leiomyoma. Accurate pretreatment prediction of the effect of GnRH analogues and ulipristal acetate[34–36] on leiomyoma volume reduction would enable the selection of patients likely to benefit from treatment and prevent surgery delay and adverse drug reactions (eg, menopause symptoms and decreased bone density), leading to an overall reduction in medical economic burden. Other common drugs, such as ulipristal acetate, may also present differences in leiomyoma volume reduction with or without *MED12* MUTs; however, ulipristal acetate is not approved for clinical use in Japan, and so it was not included in this study.

In patients with multiple uterine leiomyomas, genetic heterogeneity may arise from different MUTs.[37] These tumors have traditionally been considered in the same general category of "uterine leiomyoma" as per ultrasound imaging- and MRI-based diagnosis. Because an individual patient may have both *MED12* MUT and *MED12* WT leiomyomas, it is desirable to assess each leiomyoma or choose the leiomyoma that may be causing symptoms. If the target leiomyoma is not likely to benefit from GnRH agonist treatment based on our MRI-based classification, other medications or prompt surgical treatment may be selected.

Furthermore, *MED12* MUT leiomyomas are smaller and more often located in the subserosal part than are *MED12* WT leiomyomas.[38] The accuracy of prediction based on the *MED12* MUT status can be improved by considering the size, tumor location, multifocality, and race.[39]

Research implications

Only 3 of 56 patients in the high group had *MED12* MUTs, suggesting that

GnRH agonists would help reduce the volume of most leiomyomas in this group, whereas the reduction rate of the low group was expected to be lower because of the high proportion of *MED12* MUT leiomyomas. However, the imperfection of our MRI-based classification is in predicting *MED12* MUT in the intermediate group, which constituted 26% of the population in the analyses with a nearly equal mixture of *MED12* WT and *MED12* MUT leiomyomas, without a significant OR for prediction.

The high group had significantly higher EPO expression than the low group, reflecting that the *MED12* WT leiomyomas had higher EPO levels than the *MED12* MUT leiomyomas. We previously reported that EPO mRNA levels were correlated with tumor diameter and vessel maturity.[28] Furthermore, *MED12* WT leiomyomas had higher EPO levels and elevated EPO expression on estrogen exposure, whereas *MED12* MUT leiomyomas had lower EPO levels and did not respond to estrogen.[29] We assumed that estrogen induces EPO expression, leading to mature blood vessel development in the tumor in *MED12* WT leiomyomas and that GnRH agonists inhibit this mechanism to decrease tumor volume. Moreover, estrogen suppression does not eliminate EPO expression.[29] There may be other mechanisms leading to enhanced volume reduction in *MED12* WT leiomyomas, which should be studied further.

The 2 most common gene alterations in leiomyoma have been reported to be *MED12* MUTs and various chromosomal rearrangements related to high mobility group AT-hook 2 (HMGA2) overexpression.[40] As for the gene alteration in the high group, we suspect that most of the leiomyomas in this group carry chromosomal alteration associated with HMGA2 because these alterations and *MED12* MUT usually do not coexist in the same leiomyoma. We intended to analyze all gene alterations in this group; however, we could not afford the cost of comprehensive gene analyses. Alternatively, we confirmed the high expression of HMGA2 mRNA exclusively in the high group (data not shown). We plan to analyze the specific gene alterations within the high group in the future, which may lead us to better understand the mechanism of leiomyoma progression and/or response to treatment.

Strengths and limitations

Our study focused on the analysis of the presence or absence of *MED12* MUTs as a noninvasive predictive marker of the effect of GnRH agonists on uterine leiomyoma volume reduction. The proposed method has potential applications for predicting leiomyoma volume reduction after menopause and during GnRH agonist treatment. The main limitation of this study was that we only used leiomyoma tissue specimens for molecular analysis, and therefore, only patients for whom surgical specimens were obtained could be included. Patients who were followed up on an outpatient basis without undergoing surgery and those who were only medically treated were excluded. Some patients could avoid surgery because of a significant reduction in leiomyoma volume via GnRH agonist therapy. Here, the patient characteristics may differ from those in other studies reporting *MED12* MUT frequency.[23,26,38–41] In addition, most patients were nonobese, and all patients were of Asian ethnicity. The morbidity and progression of leiomyoma are related to race and obesity, and therefore, our method needs to be validated in a multiethnic cohort before clinical adaptation.

Conclusions

Here, we identified the differences between the characteristics of *MED12* MUT and WT leiomyomas. The *MED12* MUT status may be determined using our newly developed non–contrast-enhanced MRI-based classification system, without invasive tests, such as tissue biopsy. Thus, the *MED12* MUT status can be a candidate marker for predicting the effects of GnRH agonists.

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SUPPLEMENTAL TABLE

Summary of *MED12* mutations observed in the series of 168 uterine leiomyomas

Location	Nucleotide change	Predicted amino acid change	Number of cases (n=168)	Percentage of total
Exon 2	c.131G>A	p.G44D	18	10.7
Exon 2	c.131G>T	p.G44V	15	8.9
Exon 2	c.130G>A	p.G44S	9	5.4
Exon 2	c.130G>C	p.G44R	7	4.2
Exon 2	c.130G>T	p.G44C	4	2.4
Exon 2	c.131G>C	p.G44A	4	2.4
Exon 2	c.107T>G	p.L36R	2	1.2
Exon 2	c.128A>C	p.Q43P	2	1.2
Intron 1	c.100-8T>A	p.E33_D34delinsPQ	4	2.4
Intron 1 and exon 2	c.100-71_133del	p.D34_F45del	2	1.2
Exon 2	c.100_141del	p.D34_N47del	1	0.6
Exon 2	c.100_108del	p.D34_L36del	1	0.6
Exon 2	c.111_122del	p.A38_V41del	1	0.6
Exon 2	c.120_125del	p.N40_V41del	1	0.6
Exon 2	c.120_128del	p.N40_K42del	1	0.6
Exon 2	c.119_123delinsTT, c.181_182delinsGC	p.N40_V41delinsI, p.N61A	1	0.6
Exon 2	c.120_122del	p.V41del	1	0.6
Exon 2	c.123_131del	p.K42_G44del	1	0.6
Exon 2	c.126_137del	p.K42_F45del	1	0.6
Exon 2	c.126_131del	p.Q43_G44delinsN	1	0.6
Exon 2	c.133_162del	p.F45_D54del	1	0.6
Exon 2	c.144_167del	p.Q48_E55del	1	0.6
Exon 2	c.143_157del	p.P49_G53del	1	0.6
Exon 2	c.149_163del	p.A50_D54del	1	0.6
Exon 2	c.130_131delinsAT	p.G44I	1	0.6

MED12, mediator complex subunit 12.

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論文目録

Ⅰ　主論文

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