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MED12 and *HMGA2* mutations: two independent genetic events in uterine leiomyoma and leiomyosarcoma

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Recent identification of somatic *MED12* mutations in most uterine leiomyomas brings a new venue for the study of the tumorigenesis of leiomyomas. We are particularly interested in the correlation of *MED12* and *HMGA2* gene products in leiomyomas and leiomyosarcomas with and without *MED12* mutations. To address these issues, in this study we examined *MED12* mutations in a large cohort of usual type leiomyomas (178 cases) and uterine leiomyosarcomas (32 cases). We found that 74.7% (133/178) of leiomyomas had *MED12* mutations, which was consistent with several independent studies. In contrast, only 9.7% (3/32) of leiomyosarcomas harbored *MED12* mutations. Expression analysis by western blot and immunohistochemistry revealed that those leiomyomas with complex *MED12* mutations had significantly lower protein products than the matched myometrium. Interestingly, most leiomyosarcomas without *MED12* mutations also had very low levels of *MED12* expression in comparison to the matched myometrium. These findings suggest a potential functional role of *MED12* in both benign and malignant uterine smooth muscle tumors. When we further examined *HMGA2* expression in all leiomyomas and leiomyosarcomas, we found that *HMGA2* overexpression was exclusively present in those leiomyomas with no *MED12* mutation, accounting for 10.1% (18/178) of total leiomyomas and 40% (18/45) of non-*MED12* mutant leiomyomas. Twenty-five percent (8/32) of leiomyosarcomas had *HMGA2* overexpression, and no *MED12* mutations were found in *HMGA2* positive leiomyosarcoma. These findings strongly suggest that *MED12* mutations and *HMGA2* overexpression are independent genetic events that occur in leiomyomas, and they may act differently in the tumorigenesis of uterine leiomyomas.
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The causes of uterine leiomyomas have been investigated for many years. Although some genetic mutations have been identified in a small fraction of leiomyomas,[1–5] some of them have been confirmed to be primary genetic events specifically of germline fumarate hydratase (FH) mutations.[6] It was not until recently that Mäkinen *et al*,[7] identified a high rate of somatic gene mutations in the mediator complex subunit 12(*MED12*) gene, based on the whole exome sequencing analysis. This group further examined a total of 207 leiomyomas and nearly 70% of them had *MED12* mutations.[7] Immediately following this discovery, two additional large cohort mutation analyses in human uterine leiomyomas were conducted and had similar findings.[8,9] Current studies reveal that *MED12* mutations/variants in leiomyomas: (1) occur with a high frequency (up to 70% of tumors harbor *MED12* mutations); (2) are site-specific (71% of mutations are in codon 44, exon 2 of *MED12*); and (3) are heterozygous with no mutation detected in the normal myometrium.

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In a study of 1862 tumor samples of mesenchymal and epithelial neoplasms, *MED12* mutations/variants are specific to uterine leiomyomas and are rarely found in epithelial neoplasms.[10] As uterine smooth muscle tumors consist of a group of heterogeneous tumors, including different histological variants, the role of *MED12* mutations in different types of uterine smooth muscle tumors is interesting but remains largely unknown. Examination of *MED12* mutations in different uterine smooth muscle tumors[9,11] may help to disclose the relationship between usual type leiomyomas and other histologic variants or their malignant counterpart. Leiomyosarcomas are traditionally considered to arise *de novo*; however, recent studies suggest that some leiomyosarcomas may arise from existing leiomyomas.[12,13] The latter hypothesis is attractive, but still lacks direct molecular support. Findings of *MED12* mutations in 2–20% of uterine leiomyosarcoma[11,14–17] suggest that at least some leiomyosarcoma share the same sequence of *MED12* mutations.

About one third of uterine leiomyomas have no *MED12* mutations, and we want to study the role of *HMGA2* in leiomyomas without *MED12* mutations. We also want to know whether *MED12* mutations can be used as a biomarker for differential diagnosis. To address these issues, we first examined the spectrum of *MED12* mutations in a large cohort of benign and malignant uterine smooth muscle tumors. Then, we compared the correlation of gene mutations with the protein expression and further analyzed the patterns between *MED12* mutations and *HMGA2* expression in all of the tumors. Our results indicate that *MED12* mutations are common in benign but much less common in malignant uterine smooth muscle tumors. In particular, we have demonstrated that genetic mutations of *MED12* and *HMGA2* do not overlap in any given leiomyoma; therefore, our findings strongly suggest the presence of two independent genetic pathways in the tumorigenesis of uterine leiomyomas.

Table 1 General information of 210 uterine smooth muscle tumors in this study

	Scale and range	
Title	*Leiomyosarcoma*	*Usual leiomyoma*
No. cases	32	178
Age		
Range	47–86	22–56
Mean ± s.d.	60 ± 2	43 ± 6
Median	57	43
Tumor size (cm)		
Range	2.3–18.6	7 – 21
Mean ± s.d.	8.2 ± 0.9	11.9 ± 0.8
Median	6.9	11.3

Materials and methods

Case Selections

The use of human tissue specimens was approved by the Institutional Review Board for Human Research at Northwestern University, Chicago, IL. Fresh frozen and/or formalin-fixed and paraffin-embedded tumor, and myometrial tissues were obtained from female patients (range: 22–56 years) undergoing hysterectomies or myomectomies at the Prentice Women's Hospital in Chicago IL. Before surgery, written informed consent and demographic/endocrine information (eg, race, age, day of menstrual cycle, parity, whether oral contraception is being taken) was obtained. A total of 178 cases with histologic diagnosis of usual type leiomyoma and 32 cases with uterine leiomyosarcoma were selected for the study. The diagnoses of leiomyosarcomas were based on Bell criteria[18] and clinical presentation. Patient's clinical biodemography and pathological features were summarized in Table 1.

Immunofluorescence and Confocal Image

Immunofluorescence was performed as previously described.[19] Briefly, human myometrium and leiomyoma tissues were fixed in a modified Davidson's solution (Electron Microscopy Science, Hatfield, PA), embedded in paraffin, and sectioned at 5 μm. Anti-MED12 (1:200, Proteintech Europe, Manchester, UK) and αSMA (1:50, Abcam, Cambridge, MA) were used as primary antibodies. Alexa Fluro 488 goat anti-mouse and Alexa Fluro 594 goat anti-rabbit (Molecular Probes/Invitrogen) were used as secondary antibodies. For the counterstaining, sections were incubated with 150 ng/ml DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) (Sigma-Aldrich) for 5 min at room temperature. Confocal images were obtained with an Olympus Fluoview FV10i Confocal Microscope system (Olympus America Inc, Center Valley, PA).

Tissue Microarray and Immunohistochemistry

Tissue cores were collected from tumors and myometrial controls of each case for tissue microarray were represented in duplicate. Antibodies used for this study included HMGA2 (1:500, BioChem Inc, CA, USA) and Anti-MED12 (1:200, Proteintech Europe, Manchester, UK). Tissue microarrays were sectioned, 4 μm in thickness. After deparaffinization and antigen retrieval, all immunohistochemical staining was performed on a Ventana Nexus automated system (Tucson, AZ, USA). In brief, the endogenous peroxidase activity was blocked with 3% hydrogen peroxide. Primary antibodies were detected using standard biotinylated anti-mouse or anti-rabbit secondary antibodies. Semi-quantative immunointensity was scored as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong) and

percentage was scored as 0(0%), 1(≤10%), 2 (11–50%), 3(51–80%) ,and 4(>80%). Immunoreactivity for MED12 was scored for intensity only as it is diffusely expressed in all positive cases. The majority of HMGA2 positive cases showed strong and diffuse immunoreactivity for HMGA2, and only those cases with weak or faint immunoreactivity (intensity score 1) for HMGA2 showed a patchy staining pattern. Therefore, a combined intensity and percentage of immunoreactivity for HMGA2 were used. Moderate to high immunoreactivity (≥2) for HMGA2 in >50% of nuclei was read as HMGA2 positive.

Western Blotting

Freshly frozen myometrium and leiomyoma tissues were homogenized at 4 °C in a protein lysis buffer. Total cellular proteins from cultured cell samples were prepared with a modified radioimmunoprecipitation assay buffer, as described previously.[20] Protein concentrations were determined with the BCA protein assay kit (Pierce Protein Research Products, Rockford, IL, USA). The cell lysates (40 μg of total protein per sample) were separated on 8% sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gels. After electrophoresis, the proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore Corp, Bedford, MA, USA). The blotted membranes were blocked for 1 h in a Tris-buffered saline with 5% non-fat milk at room temperature and subsequently incubated with primary antibodies against the proteins of interest. The rabbit anti-human MED12 was obtained from Bethyl Laboratory (Montgomery, TX, USA). After incubation with the primary antibody, the PVDF membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibodies (goat anti-rabbit, Amersham Biosciences, Piscataway, NJ, USA). Immune complexes were detected on the membranes with a chemiluminescent reagent (GE Healthcare Biosciences, Piscataway, NJ, USA). An anti-β-actin monoclonal antibody (Abcam, Cambridge, MA, USA) and an anti-α-smooth muscle actin (Abcam) or anti-GAPDH (Cell signaling technology, Danvers, MA, USA) were used as a loading control. Net intensities for the protein bands of interest were determined using Adobe Photoshop. Band intensities were then normalized to β-actin intensities and compared for differences between experimental and control conditions.

DNA Extraction from Fresh Frozen and Formalin-Fixed and Paraffin-Embedded Tissue

We used the Qiagen DNeasy Blood and Tissue kit and the protocols for fresh frozen and formalin-fixed tissues. For formalin-fixed and paraffin-embedded tissue, six to eight 10 μm tissue sections were collected into 1.5 ml Ependoff tubes. Paraffin was dissolved in xylene and removed. Tissue samples were then lysed under denaturing conditions with a proteinase K digestion at 56 °C for 1 h, followed by incubation at 90 °C to reverse formalin cross-linking. DNA was purified by column purification with a filter membrane and stored in −20 °C before use.

PCR and Sequencing

For PCR amplification, 50 ng of genomic DNA was used. Primers used for the amplification of MED12 exon 2 and flanking the exon–intron junction sequence were 5′- GCC CTT TCA CCT TGT TCC TT-3′ (forward) and 5′-TGT CCC TAT AAG TCT TCC CAA CC-3′ (reverse). PCR products were purified by ExoSAP-IT reagent (Affymetrix, Inc) following the manufacturers instruction. DNA sequencing of the purified DNA products was performed by GENEWIZ, Inc (South Plainfield, NJ, USA) and Genomic Core Facility at the Northwestern University using Applied Biosystems BigDye version 3.1. The reactions were then run on Applied Biosystem's 3730xl DNA Analyzer. Mutations/variations were analyzed by the DNASTAR Lasergene 9 software for analysis.

Statistical Analysis

Gene expression levels by immunohistochemistry were summarized by medians and ranges for the entire patient population, and separated by tumors and matched myometrium, and histology and clinical groups. Differences between tumors and matched myometrium, and among histology and clinical groups were assessed using Wilcoxon rank-sum tests. Associations among the gene expression levels were assessed via the Spearman correlation. P values less than 0.05 ($P<0.05$) were considered statistically significant.

Results

MED12 Mutations in Usual Type Leiomyomas and Leiomyosarcomas

We collected 178 uterine leiomyomas, and all usual type reviewed and confirmed by histology examination. Tumor size ranged from 3 to 21 cm. Patients' age ranged from 22 to 56 years with a median age of 42 years old (Table 1). Among them, 64 were black women, 52 white women, 13 Hispanic, and four Asian women (Table 2).

Of all 178 leiomyomas examined, 74.7%(133/178) of uterine leiomyomas had *MED12* mutations. Among them, 93.9%(125/133) of mutations were confined to exon 2 and the remaining 6.1% in intron 1. For those leiomyomas with *MED12* mutations, point mutations were found in 87.9%(117/133) of cases and complex *MED12* mutations in 12.1%(16/133). Overall, point mutations spread to 12-single nucleotide sites, and the most common point

mutations were found in two nucleotide sites of c130 and c131, accounting for 72.9%(97/133) of *MED12* mutations (Figure 1, Table 1). Leiomyomas with *MED12* mutations seemed to be evenly distributed among different races, and no racial difference was noted (Table 1). This is consistent with another recent study.[9] No significant associations of *MED12* mutations with tumor size and patients age were seen.

Table 2 *MED12* mutation analysis in different types of uterine smooth muscle tumors

	MED12 mutations		
Tumor types	*No. positive*	*No. negative*	*% of mutant cases*
Leiomyosarcomas	3	29	9.4%
Usual leiomyomas	134	43	75.7%
Usual leiomyomas			
Black women	64	17	79.0%
White women	53	21	71.6%
Hispanic women	13	3	81.3%
Asian women	4	2	66.7%

A total of 32 uterine leiomyosarcomas were included in this study. Patients age ranged from 47 to 86 years old and the tumor size of 2.3–18.6 cm (Table 1). Fifty-nine percent of them were stage I and the rest of the cases were II–IV. Only three cases presented with *MED12* mutations accounting for 9.4% of the tumor population (3/32). Of three cases with *MED12* mutations, one had a deletion of 39 nucleotides, corresponding to 13 amino acids shorter, one had a frame-shift likely resulting in no protein product, and one had a point mutation of G–A change at a position of 131 (Figure 2). Findings suggest that *MED12* mutations in uterine leiomyosarcomas are very low.

Expression Analysis of Wild Type and Mutant MED12 in Leiomyomas and Leiomyosarcomas

The correlation of *MED12* mutations and its gene product in leiomyoma remains unclear. Studying *MED12* gene products will not only help us to understand the function of this gene but will also provide a potential biomarker for clinical application, as well as for differential diagnosis. To this

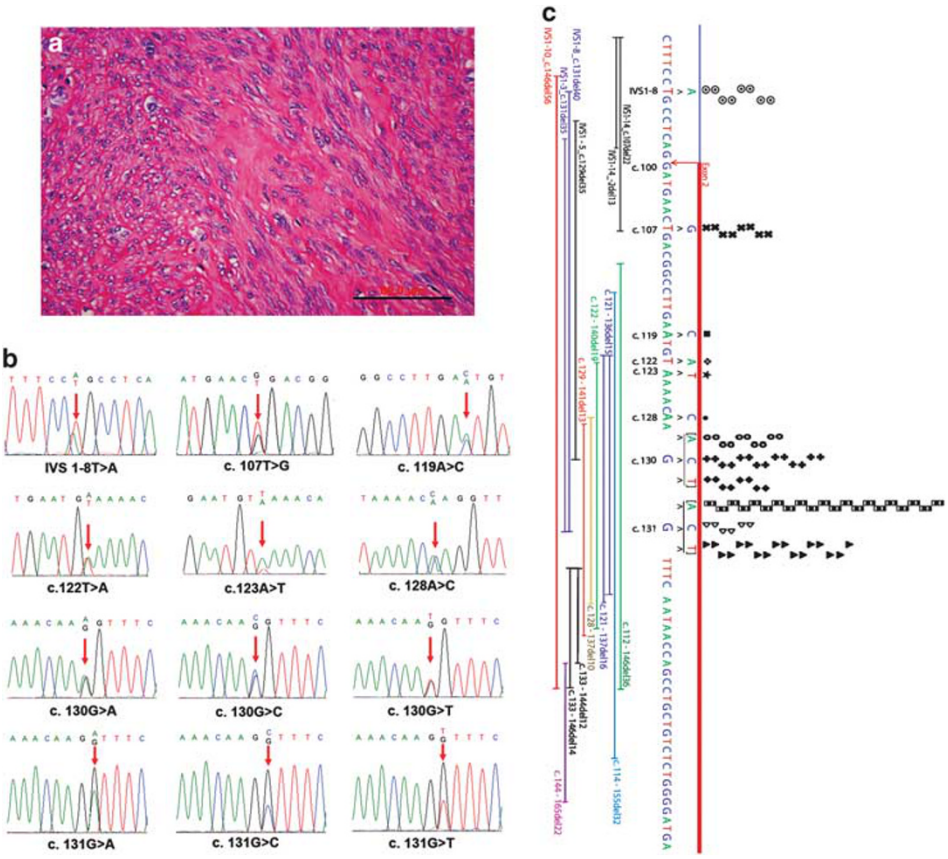

Figure 1 *MED12* mutation analysis in uterine leiomyomas. (**a**) The photomicrograph illustrates the histology of usual type leiomyoma. (**b**) Sequencing chromatographs of 12 types of *MED12* mutations identified in usual type leiomyomas. Red arrows indicate the mutation sites. (**c**) Genomic analysis of the types and distribution of *MED12* mutations in exon2 and intron1. The point mutations were listed in the above sequences and each dot represents a case with mutation. The complex mutations were listed below the sequence with thin bars illustrating the actual length of deletions.

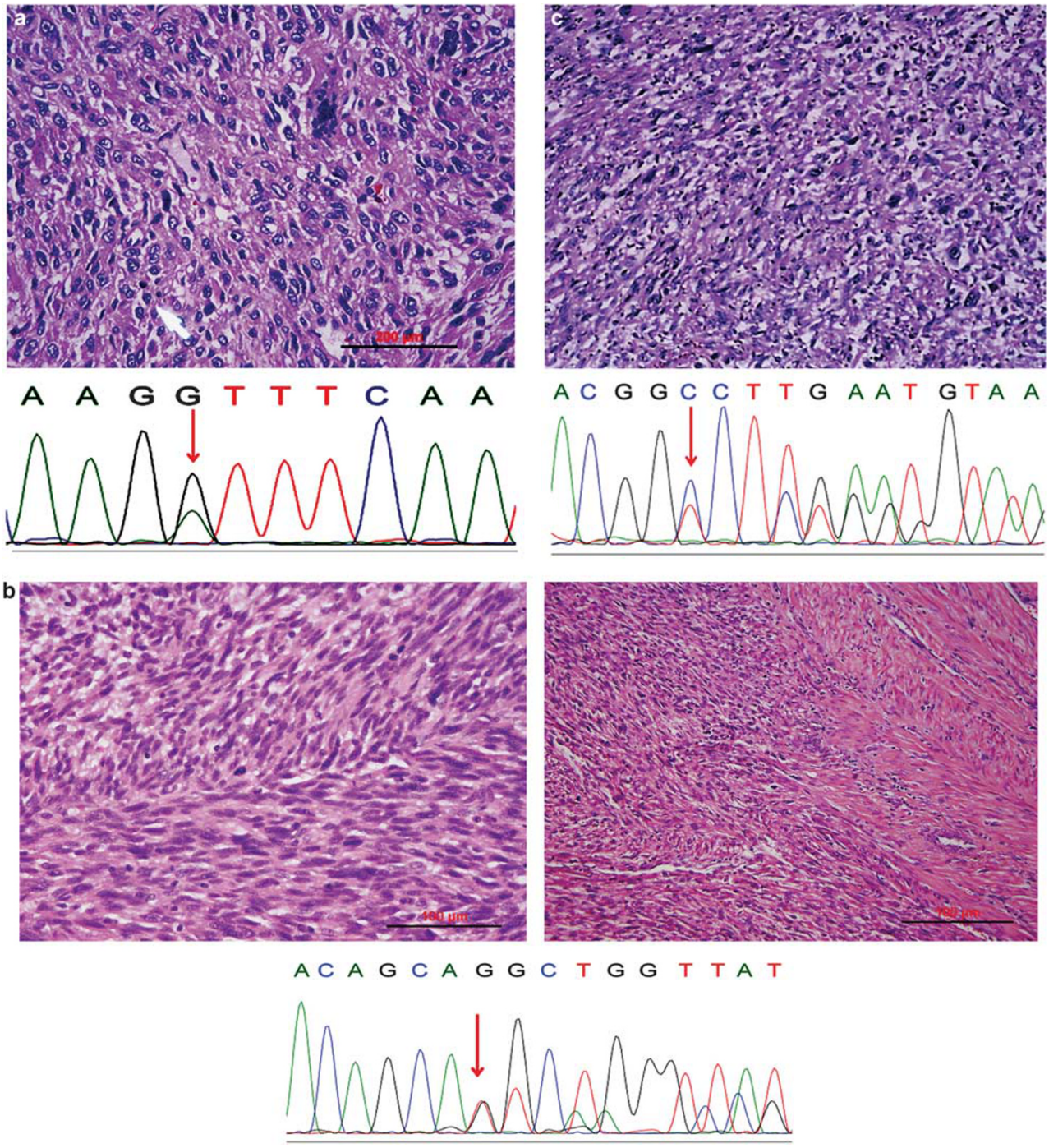

Figure 2 Three leiomyosarcomas with *MED12* mutations. (**a–c**). Illustrate the high power histology of three leiomyosarcomas (upper) and their corresponding sequencing chromatographs for *MED12* mutations (lower). Red arrows indicate the mutation sites. Case two (**b**) is a leiomyosarcoma of stage IV disease.

end, we examined *MED12* gene products by western blot in the selected leiomyomas with wild type, simple, and complex *MED12* mutations. Simple mutations were defined as point mutations involving a single nucleotide replacement, whereas complex *MED12* mutations referred to those changes of insertion, deletion, and shifting of the coding sequence (Figure 1). We found that leiomyomas with a complex *MED12* mutation seemed to have very low or a complete loss of its gene product, and those tumors with simple *MED12* mutations showed slightly reduced protein expression (Figure 3). These findings were further supported by the immunofluorescent stain of MED12. With the aid of internal counterstains of DAPI and smooth muscle actin-α, loss of MED12 expression

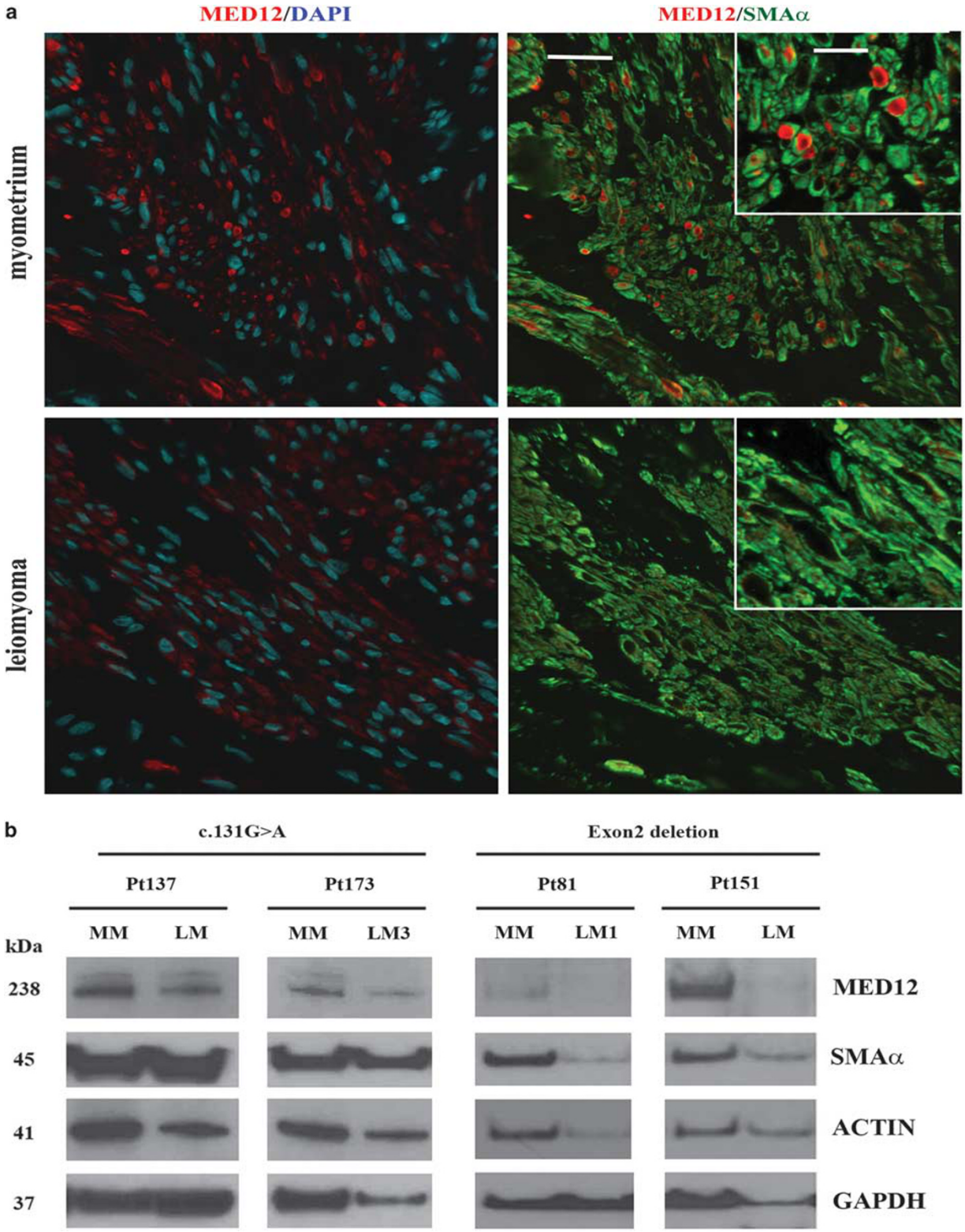

Figure 3 Reduced MED12 protein levels in uterine leiomyomas with complex MED12 mutations. (**a**) Confocal microscopic images illustrate an example of immunofluorescent staining for MED12 (red), smooth muscle actin (green), and DAPI (blue) in leiomyoma with a complex MED12 mutation (with a partial MED12 Exon 2 deletion) and its matched myometrium. In myometrium, the expression pattern and distribution are punctuated with high intensity staining enriched in perinuclear localization. In contrast, the expression pattern for leiomyoma is diffused in the cytoplasm and has lesser staining intensity. Scale bars = 50 μm. Higher power image of MED12 staining in leiomyoma and myometrium are shown in the insert (Scale bar = 25 μm). (**b**) The expression of MED12 protein was detected by western blot analysis in leiomyomas with complex MED12 mutations (Pt81LM and Pt151LM for Exon 2 deletion on right) and leiomyomas with simple MED12 point mutations (Pt137LM and Pt173LM for c.131G>A on left). MED12 expression in the matched myometrium (MM) was shown next to tumors. Anti-α-smooth muscle actin, Anti-Actin, and anti-GAPDH were used as protein loading controls.

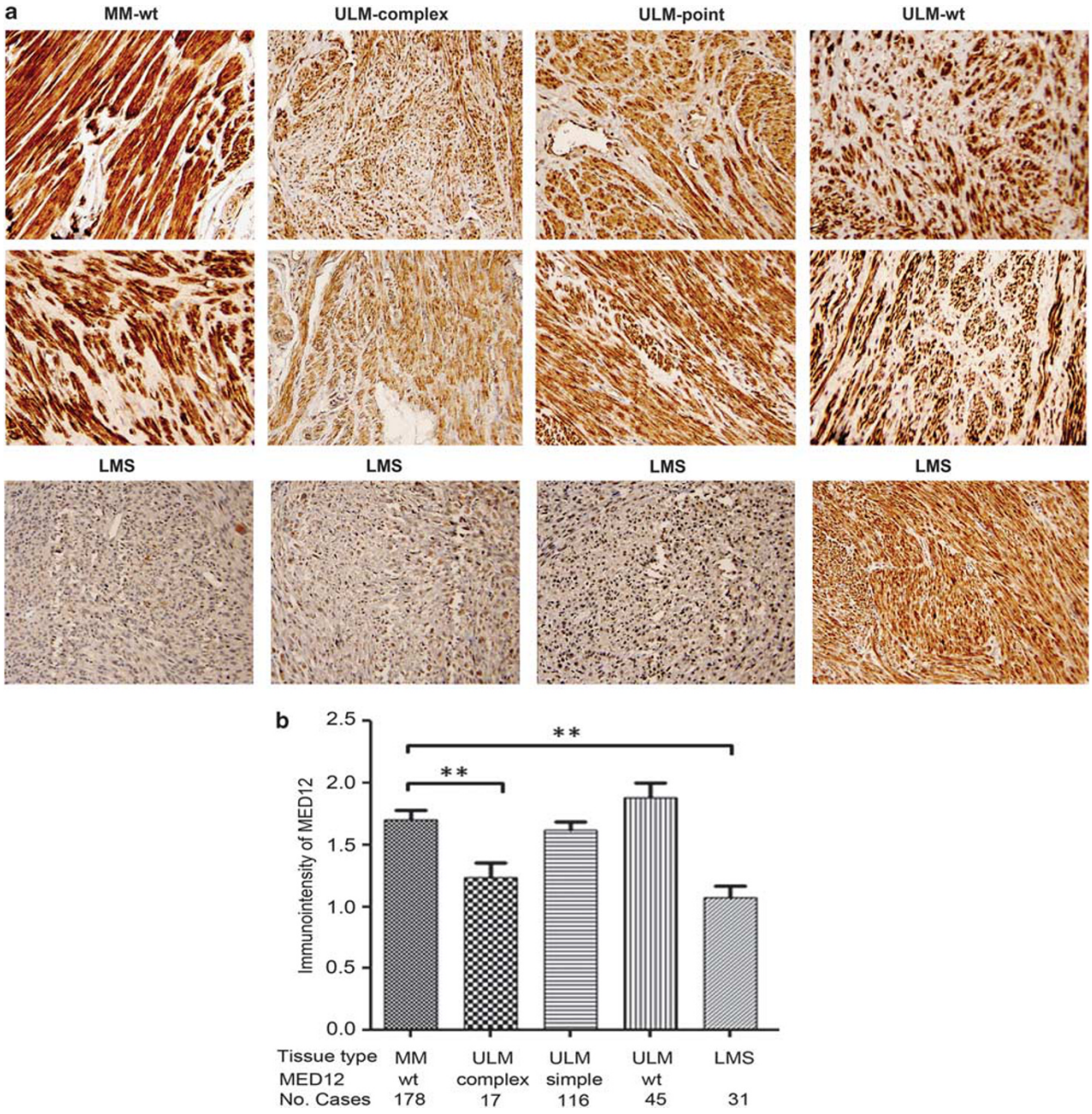

Figure 4 Immunohistochemical analysis of MED12 expression in formalin-fixed and paraffin-embedded leiomyomas and leiomyosarcomas. (**a**) Photomicrographs illustrate examples of immunointensity of MED12 in tumor myometrium (MM), leiomyomas (ULM) and leiomyosarcoma (LMS). wt: wild type *MED12*; point: point/single nucleotide mutation of *MED12*; complex: complex mutations of *MED12*. (**b**) Statistical analysis of immunointensity for MED12 in tumor myometrium (MM), leiomyomas (ULM) and leiomyosarcoma (LMS). *MED12* mutation types and number of cases were listed below the histobars. $^{**}P<0.01$.

in leiomyomas with complex *MED12* mutations was evident in comparison to its matched myometrial tissue section (Figure 3).

To explore the potential application of immunohistochemistry for *MED12* expression in a formalin-fixed and paraffin-embedded tissue, we examined MED12 expression by immunohistochemistry (see Materials and methods) in all 178 leiomyomas and matched myometrium in a tissue microarray. As immunoreactivity for MED12 seemed to be diffusely distributed in the cytoplasm with some nuclear localization, we scored immunointensity by semi-quantitative scales of negative, weak, moderate, and strong expression (Figure 4). Consistent with western blot results, we found that leiomyomas with complex *MED12* mutations had significant lower immunoreactivity for MED12 ($P<0.01$) than in myometrium. Notably, no significant difference of

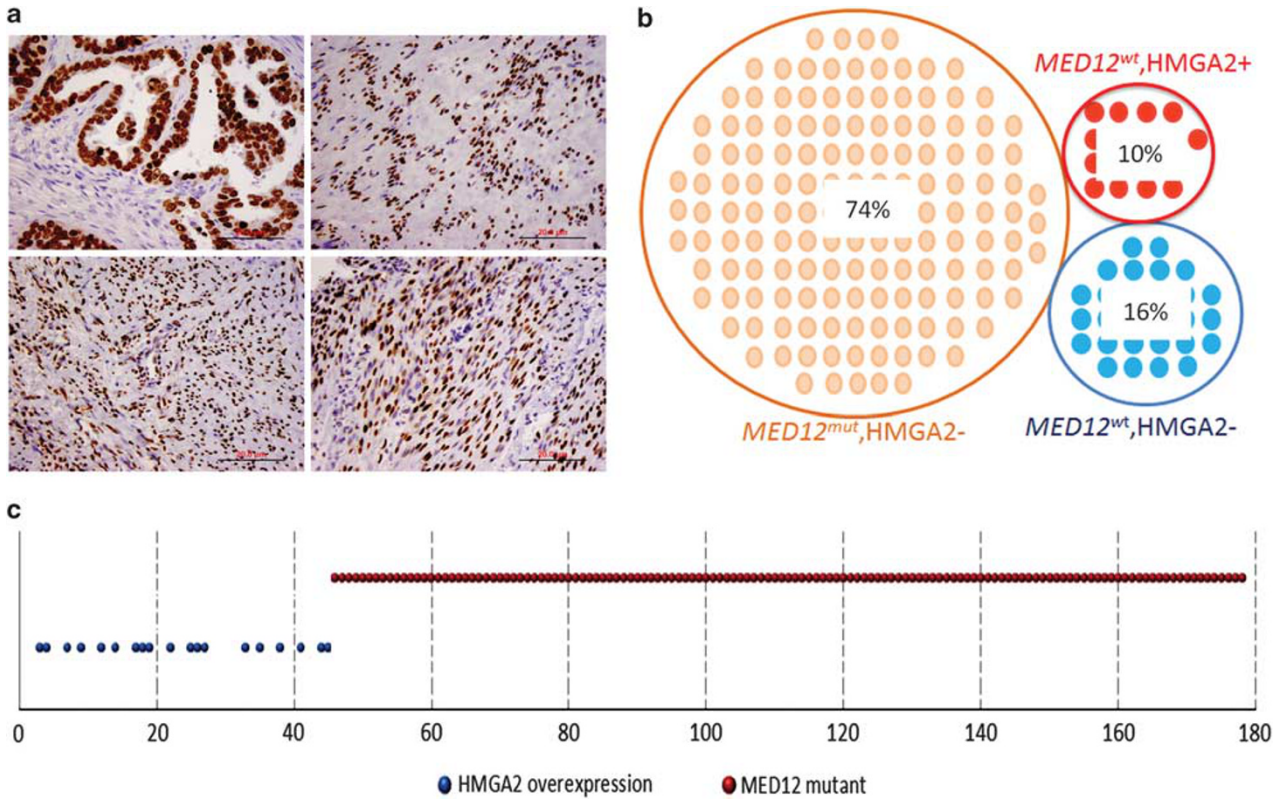

Figure 5 HMGA2 overexpression in uterine leiomyomas. (**a**) Photomicrographs illustrate examples of strong and diffuse immunoreactivity for HMGA2 in uterine leiomyomas. High grade serous ovarian carcinoma was used as the positive control (left upper). (**b**) and (**c**) Dot-plot analysis of distribution of *MED12* mutations and HMGA2 overexpression in 178 cases of uterine leiomyomas. Seventy-four percent of leiomyomas have *MED12* mutations and lack of HMGA2 overexpression; 10% of leiomyomas have wild type *MED12* and HMGA2 overexpression; and remainder 16% of leiomyomas have wild type *MED12* and lack of HMGA2 overexpression.

MED12 expression between myometrium and leiomyomas of wild type and simple *MED12* mutations was found.

As reported, only three leiomyosarcomas had *MED12* mutations. However, most leiomyosarcomas without the *MED12* mutations showed significantly lower, or were absent of, immunoreactivity for MED12 (Figure 4).

HMGA2 Expression in Leiomyomas and Leiomyosarcoma with and without MED12 Mutations

Twenty-six percent of leiomyomas had no *MED12* mutations. *HMGA2* (high mobility group A2) overexpression induced by non-random chromosomal translocation t(12;14) was found in less than 10% of leiomyomas.[21] To investigate whether *HMGA2* overexpression correlates with *MED12* mutations, we examined and compared the expression pattern of *HMGA2* with *MED12* mutations by immunohistochemistry in all 178 leiomyomas and 32 leiomyosarcomas. We found that a total of 18 leiomyomas and eight leiomyosarcomas were strongly immunoreactive for HMGA2, accounting for 10.1 and 25.0% of cases, respectively. Surprisingly, *HMGA2* overexpression was exclusively found in those leiomyomas without a *MED12* mutation (Figure 5). To confirm this finding, we repeated the HMGA2 stain in a different lab using a new vial of HMGA2 antibody of BioCheck, and the same results were found. Consistent with the finding in leiomyomas, leiomyosarcomas with *HMGA2* overexpression did not harbor the *MED12* mutation either. Our result was further supported by a study of 38 leiomyomas, in which the *MED12* mutation and *HMGA2* overexpression were identified in completely separate tumor populations.[22]

Discussion

Uterine leiomyoma is a major public health problem. Millions of reproductive aged women suffer from these tumors, and there are roughly 200 000 hysterectomies for symptomatic leiomyomas performed annually in the United States[23] causing substantial medical costs.[24,25] Uterine leiomyomas are genetically heterogamous. Some tumors harbor non-random chromosomal translocations t(12;14) for *HMGA2* overexpression,[26,27] and rare cases have germline gene mutations in FH[28] and *COL4A5/A6*.[29] Recent identification of *MED12* mutations in most uterine leiomyomas provides a new and promising candidate gene for understanding the cause and

tumorigenesis of leiomyomas. In particular, *MED12* mutations seem to be mostly confined to uterine leiomyomas.[13,17] Although it is still too early to say that *MED12* mutations are the direct causal factor for the development of most leiomyomas, these clinically significant findings will accelerate medical research to characterize the functional role of *MED12* in the disease. Eventually, this will benefit the clinical application.

Based on several large cohorts and this study, the mutation rate of *MED12* in uterine leiomyomas varies with a range from 50 to 70% of tumor population. *MED12* mutations in uterine leiomyomas were mostly identified in exon 2 and rarely in the intron 1–exon 2 junction.[7,8] All mutations were heterozygous in genomic DNA, and all of the transcripts were derived solely from the mutant *MED12* alleles.[10,11] Therefore, in this study, we examined *MED12* gene mutations covering the exon 2 and intron 1–exon 2 junction. We found that 75% of tumors harbor *MED12* mutation (Figure 1).

Notably, at least a quarter of leiomyomas have no detected *MED12* mutations. *HMGA2* overexpression is the second most common genetic alteration in leiomyomas, identified in 7.5–10% of leiomyomas due to t(12;14) translocation. In two independent studies, a total of 118 leiomyomas with cytogenetic information,[9,22] and no *MED12* mutations in tumors with *HMGA2* overexpression were found. To further investigate the relationship of *MED12* mutations and HMGA2 overexpression, we examined *HMGA2* expression in all 178 leiomyomas and 32 leiomyosarcomas by immunohistochemistry. As illustrated in Figure 5, *HMGA2* overexpression was exclusively found in those tumors without *MED12* mutations. The current study is the largest sample size for the analysis of *MED12* mutations and *HMGA2* expression. Combining these three studies into nearly 300 cases strongly suggests that there are two mutually exclusive independent pathways of *MED12* and *HMGA2*, and they are likely playing different roles in the tumorigenesis of uterine leiomyoma.

Furthermore, although there is a low rate of *MED12* mutations in uterine leiomyosarcomas, these two genetic mutations were independent. *HMGA2* is an oncogene, and its oncogenic properties have been well characterized in many benign and malignant neoplasms. A recent study further defined that *HMGA2*-mediated tumorigenesis in leiomyomas is mainly through a regulation of the cell-cycle checkpoint in the G1/S phase.[21] In the current study, we found that *HMGA2* overexpression accounts for 45% of *MED12* negative tumors. The findings suggest that some other genetic mutations, such as *HMGA1*, *FH*, *COL4A5/A6,* and as yet to be characterized genetic factors may be responsible for the remaining leiomyomas without *HMGA2* and *MED12* mutations.

As mentioned above, *MED12* mutations are the major genetic changes in benign leiomyoma, whereas the mutations are much less common in the malignant counterpart—leiomyosarcoma. Furthermore, MED12 protein expression in leiomyomas with *MED12* mutations has only been examined by the western blot analysis in one study,[16] and no immunohistochemistry analysis for MED12 has been reported. To assess the value of immunohistochemistry for MED12 expression in formalin-fixed paraffin-embedded tissues, we compared and correlated the immunointensity of MED12 in tumors with/without *MED12* mutations. Although western blot analysis can detect the reduced MED12 protein in both simple (point) and complex mutations of the *MED12* gene (Figure 3), the immunohistochemical stain can only detect the reduced immunoreactivity for MED12 in tumors with the complex *MED12* mutations (Figure 4). More studies are needed to further optimize the immunohistochemistry stain of this important gene marker for its potential clinical application.

MED12 mutations in leiomyosarcoma have drawn great attention recently and several studies have shown that *MED12* mutations in leiomyosarcoma ranged from 3 to 20%.[11,14,17] In this study, we found that the rate of *MED12* mutations was nearly 10% in uterine leiomyosarcomas. When we combined all of the studies of leiomyosarcomas reported in the literature with our cases,[10,14–17,26] a total of 174 leiomyosarcomas, 14 tumors had *MED12* mutations, accounting for an 8.1% mutation rate. Therefore, *MED12* mutations in leiomyosarcoma are far lower than those in leiomyomas. Interestingly, two of three *MED12* mutations in our series are either large deletion or frame-shift, which are rarely seen in leiomyomas (Figure 2). Nevertheless, 9.4% of leiomyosarcomas share genetic mutations of *MED12* with leiomyomas, suggesting that a small fraction of leiomyosarcomas may progress from existing leiomyomas. This may warrant further study.

In summary, our current study of benign and malignant uterine smooth muscle tumors concludes that: (1) *MED12* mutations are common in usual type leiomyomas; (2) leiomyomas with *HMGA2* overexpression do not share or harbor *MED12* mutations; (3) complex *MED12* mutations lead to loss or reduced MED12 protein product; and (4) leiomyosarcomas have a very low rate of *MED12* mutations, suggesting that most leiomyosarcomas have independent tumorigenic pathways from their benign counterpart.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

Author contributions

Design: JJW, TK. Experiments: EB, WQ, QZ, MEF, SD, YL, JJW. Materials: JJW, TK, BHK. Analysis: JJW, WQ, TK. Manuscript: JJW.

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