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Brief Report

Genetics

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Med12 gain-of-function mutation causes leiomyomas and genomic instability

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Uterine leiomyomas are benign tumors that can cause pain, bleeding, and infertility in some women. Mediator complex subunit 12 (*MED12*) exon 2 variants are associated with uterine leiomyomas; however, the causality of *MED12* variants, their genetic mode of action, and their role in genomic instability have not been established. Here, we generated a mouse model that conditionally expresses a *Med12* missense variant (c.131G>A) in the uterus and demonstrated that this alteration alone promotes uterine leiomyoma formation and hyperplasia in both WT mice and animals harboring a uterine mesenchymal cell-specific *Med12* deletion. Compared with WT animals, expression of *Med12* c.131G>A in conditional *Med12*-KO mice resulted in earlier onset of leiomyoma lesions that were also greater in size. Moreover, leiomyomatous, *Med12* c.131G>A variant-expressing uteri developed chromosomal rearrangements. Together, our results show that the common human leiomyoma-associated *MED12* variant can cause leiomyomas in mice via a gain of function that drives genomic instability, which is frequently observed in human leiomyomas.

Introduction

Uterine leiomyomas, or fibroids, are benign tumors arising from smooth muscle cells of the uterus. They are clinically diagnosed in 25% of women of reproductive age and are often associated with dysmenorrhea, dyspareunia, menorrhagia, infertility, and miscarriages (1, 2) and are the single largest cause of hysterectomy. Leiomyomas are monoclonal in origin, and 40% of the tumors have karyotypic abnormalities, including deletions in chromosome 7, trisomy of chromosome 12, and rearrangements involving the *HMGA1* (6p21) and *HMGA2* (12q14) genes (3–5). Whole-exome approaches have identified heterozygous somatic mutations in the mediator complex subunit 12 (*MED12*, Online Mendelian Inheritance in Man [OMIM] 300188) in approximately 70% of leiomyomas in patients from various ethnic and racial groups (6, 7). The majority of identified mutations occur in exon 2 of *MED12*.

MED12 is located on the X chromosome and encodes a 250-kDa protein that is a subunit of the large mediator complex and is involved in transcriptional regulation of the RNA polymerase II complex. The *MED12* protein is highly conserved among eukaryotes (8) and plays an important role during embryogenesis, as *Med12*-null mouse embryos arrest at E7.5 due to impaired mesoderm formation (9). Despite the high prevalence of *MED12* mutations within human uterine leiomyomas, their causality and mode of action are not well understood. Here, we show that the common *Med12* variant associated with human leiomyomas, *Med12* c.131G>A, can drive tumor formation alone in a gain-of-function manner and causes genomic instability.

Results and Discussion

Conditional loss of function of Med12 does not lead to uterine hyperplasia or leiomyomas. We first determined whether the conditional inactivation of *Med12* causes leiomyomas. Since *Med12* is expressed from the X chromosome, random X chromosome inactivation will lead to random expression of either the paternal or maternal *Med12* locus in uterine myometrial cells. We crossed anti-Müllerian hormone receptor type II-driven Cre (*Amhr2-Cre*) (10) with *Med12*^{fl/fl} animals (9) to generate *Med12*^{fl/+} *Amhr2-Cre* animals and studied the effects of *Med12* deficiency in a subpopulation of uterine mesenchymal cells. The use of *Med12*^{fl/+} animals, in which 1 allele is floxed and the other is WT, allowed us, in the presence of *Amhr2-Cre* recombinase, to generate a mosaic population of cells that either express or lack *Med12*.

Since *Amhr2-Cre* acts well after X chromosome inactivation is established (E6.5) (11), loss of *Med12* function will not lead to skewed X inactivation in mouse uteri. To assess the Cre recombination in our hands, we crossed *Amhr2-Cre* mice with double-fluorescent Cre-reporter *mT/mG* mice (12), which express red fluorescence in all tissues and green fluorescence upon Cre recombination. Given our results (Supplemental Figure 1, A and B; supplemental material available online with this article; doi:10.1172/JCI81534DS1), we determined that approximately 60% of uterine mesenchymal cells underwent Cre-mediated excision. Recombination of the *Med12*^{fl} allele and reduction of *Med12* mRNA transcripts were confirmed in *Med12*^{fl/+} *Amhr2-Cre* uteri (Supplemental Figure 1, C–E). Neither leiomyoma formation nor hyperplasia were observed in adult uteri of *Med12*^{fl/+} *Amhr2-Cre* mice (Supplemental Figure 1, G and I). These results indicate that *Med12* loss of function is not a mechanism of leiomyoma formation.

Expression of the Med12 c.131G>A variant on a background of conditional Med12 KO causes leiomyomas. The most common *MED12* mutation in leiomyomas among American women is a

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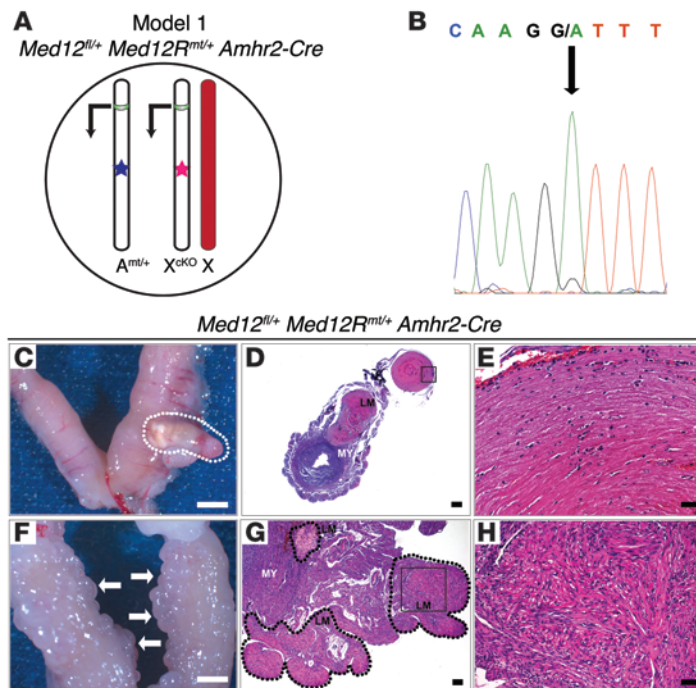


Figure 1. *Med12^{fl/fl}* *Med12^{Rmt/+}* *Amhr2-Cre* mice develop uterine leiomyomas. (A) Mouse model 1 (*Med12^{fl/fl}* *Med12^{Rmt/+}* *Amhr2-Cre*). A subset of cells express the *Med12* c.131G>A variant from the autosomal *ROSA* locus, while X chromosome-derived *Med12* will either be conditionally excised at 1 locus, or silenced by X chromosome inactivation at the other locus. Transcription from the mutant autosome (*A^{mt/+}*) is indicated with an arrow, and the promoter region is depicted in green. The blue star indicates the *Med12* c.131G>A variant. The pink star indicates the floxed *Med12* allele on the X chromosome (*X^{cko}*), which, in the presence of *Amhr2-Cre*, will lose exons 1–7. In the cells in which the *Med12* floxed allele is inactivated, WT *Med12* will be expressed. In cells with an active *Med12* floxed allele, only mutant *Med12* will be expressed. The red chromosome indicates the inactivated X chromosome. (B) cDNA sequencing from tumor tissue showing the presence of the mutant c.131G>A variant (green chromatogram peak, black arrow) in the absence of the WT *Med12* allele, consistent with the monoclonal composition of tumor cells. (C) Eighteen-week-old multiparous *Med12^{fl/fl}* *Med12^{Rmt/+}* *Amhr2-Cre* uterus revealed a 4-mm tumorous lesion (outlined by white dotted lines). (D) Histological examination confirmed the presence of a large leiomyoma nodule growing from the smooth muscle layer of the uterus. A higher-magnification image of the black-boxed neoplastic area appears in E and shows the presence of fascicles with plump spindle cells, eosinophilic cytoplasm, and ECM deposits. (F) Twenty-four-week-old *Med12^{fl/fl}* *Med12^{Rmt/+}* *Amhr2-Cre* multiparous female uterus showing multiple nodules (white arrows). (G) Multiple leiomyoma nodules are outlined by black dotted lines, and the black box, shown at higher magnification in H, highlights fibrosis and ECM deposition. Approximately 80% (16 of 20 females) of the uteri exhibited leiomyoma-like lesions and hyperplasia. LM, leiomyoma; ES, endometrial stroma; MY, myometrium. Scale bars: 2,000 μm (C and F), 1,000 μm (D), 500 μm (G), 100 μm (H), 50 μm (E).

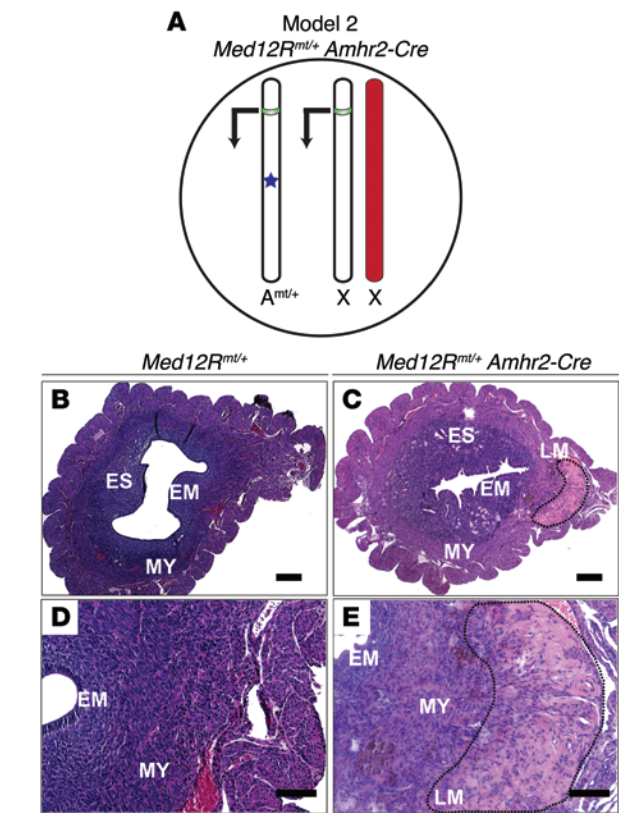
nonsynonymous variant, c.131G>A, predicted to substitute a highly conserved glycine with aspartic amino acid (p.Gly44Asp) (7). We investigated whether this *Med12* mutation causes leiomyoma formation by generating a floxed *Med12*-mutant knockin mouse model (Supplemental Figure 2, A and B). We engineered the c.131G>A variant into the mouse *Med12* cDNA (*Med12^{mt}*) fused with a FLAG tag, subcloned it into the pROSA26-DV1 vector, and integrated it into the autosomal *ROSA26* genomic locus. The presence of the FLAG reporter allowed us to distinguish the expression of mutant *Med12* from WT *Med12* (Supplemental Fig-

ure 2, D and F). The mice generated were heterozygous for mutant *Med12* cDNA at the *ROSA26* locus (*Med12^{Rmt/+}*). We mated *Med12^{Rmt/+}* mice with *Amhr2-Cre* mice to conditionally express the mutated *Med12* (c.131G>A) as early as E13.5 in the mouse uterine mesenchyme (10).

We investigated whether uterine leiomyomas will form in mice that express the *Med12* c.131G>A variant on a conditional KO background (Figure 1A). In this model, *Med12^{fl/y}* *Amhr2-Cre* males were bred with *Med12^{Rmt/+}* females to generate *Med12^{fl/+}* *Med12^{Rmt/+}* *Amhr2-Cre* females. A subset of uterine cells will express *Med12* c.131G>A on an X chromosome *Med12*-null background (Figure 1B). We analyzed the *Med12^{fl/+}* *Med12^{Rmt/+}* *Amhr2-Cre* female reproductive tracts at 8 weeks and beyond 12 weeks of age. Nulliparous *Med12^{fl/+}* *Med12^{Rmt/+}* *Amhr2-Cre* female mice presented with pathological changes associated with leiomyoma formation as early as 8 weeks of age (Supplemental Figure 3, B and D). Histological evaluation revealed that, beyond 12 weeks of age, 80% of the uteri contained lesions consistent with leiomyomas. These lesions consisted of extracellular matrix (ECM) deposits, accompanied by infiltration of fibroblasts and macrophages, hyperplasia, and disorganized muscle fiber arrangement, leading to complete destruction of myometrial architecture. Tumors formed in *Med12^{fl/+}* *Med12^{Rmt/+}* *Amhr2-Cre*-mutant uteri expressed mutant *Med12*, as shown by the expression of FLAG, which was fused to mutant *Med12* in our *ROSA* construct (Supplemental Figure 2F).

It has been noticed that estrogen and progesterone promote leiomyomatous growth, and 30% of leiomyomas in human pregnancies increase in volume (13). To corroborate these observations, we studied the effects of mouse parturition on leiomyomatous growth. Multiparous *Med12^{fl/+}* *Med12^{Rmt/+}* *Amhr2-Cre* females often had either grossly visible large leiomyomas (Figure 1C) or multiple small leiomyoma-like nodules (Figure 1F). Histology confirmed that these tumors arose from the smooth muscle layer and consisted of whorled fascicles of fusiform smooth muscle cells with an abundance of eosinophilic cytoplasm and ECM deposits (Figure 1, D, E, G, and H), consistent with the pathology seen in human uterine leiomyomas. Large tumors were often necrotic, hemorrhagic, and fibrotic. In addition, characteristic of leiomyomas, all tumors stained positive for smooth muscle actin and showed an abundance of collagen deposits when stained with Masson's trichrome stain (Supplemental Figure 4, A–C). Eighty percent of multiparous *Med12^{fl/+}* *Med12^{Rmt/+}* *Amhr2-Cre* females had leiomyoma-like lesions. These results indicate that the *Med12* c.131G>A variant causes leiomyoma-like lesions in mice.

The *Med12* c.131G>A variant can cause uterine leiomyomas in mice on a WT background. We investigated whether leiomyoma-like lesions were also present when the *Med12* c.131G>A variant was expressed in mice on a WT background (Figure 2A). We generated animals coexpressing mutant *Med12* from the autosome and a WT *Med12* from the X chromosome (*Med12^{Rmt/+}* *Amhr2-Cre*) by crossing *Med12^{Rmt/mt}* and *Amhr2-Cre* mice. Uteri from nulliparous *Med12^{Rmt/+}* *Amhr2-Cre* and control mice (*Med12^{Rmt/+}*) were examined at 8 weeks of age and after 12 weeks of age.



In 8-week-old *Med12^{Rmt/+} Amhr2-Cre* mice, no leiomyoma-like lesions were observed (Supplemental Figure 5B). Fifty percent of the uteri from *Med12^{Rmt/+} Amhr2-Cre* mutant mice that were over 12 weeks of age showed hyperplasia and leiomyomas, characterized by ECM deposition and a disorganized pattern of smooth muscle fiber arrangement (Supplemental Figure 5D). Uteri that expressed mutant *Med12* weighed 20% to 30% more than did control uteri ($P < 0.05$) (Supplemental Figure 5E). Examination of uteri from mice that were beyond 12 weeks of age revealed nodules that histologically resembled human leiomyomas due to deposition of ECM, whorl formation, and fewer nuclei (Figure 2, C and E). Our results show that the *Med12* mis-

Figure 2. *Med12^{Rmt/+} Amhr2-Cre* uteri develop prominent leiomyomas. (A) Mouse model 2 (*Med12^{Rmt/+} Amhr2-Cre*). A subset of cells that express *Amhr2-Cre* will express the *Med12* c.131G>A variant from the autosomal *ROSA* locus in the presence of X chromosome WT *Med12*. Transcription from a mutant autosome (*A^{mt/+}*) is shown with an arrow, and the promoter region is depicted in green. The *Med12* c.131G>A variant is depicted with a blue star. The red chromosome indicates the inactivated X chromosome. (B and D) Uteri from *Med12^{Rmt/+}* control mice that, in the absence of *Amhr2-Cre*, did not express the *Med12* c.131G>A variant and showed normal cross-sectional histology. (C and E) Uteri from *Med12^{Rmt/+} Amhr2-Cre* mice that expressed the *Med12* c.131G>A variant and revealed leiomyoma-like lesions in approximately 47% (8 of 17) of the females, with a typically sparse nuclear arrangement, a nodular pattern of cellular growth, and ECM deposition (black dotted lines). EM, endometrium. Scale bars: 500 μ m (B and C), 100 μ m (D and E).

sense variant c.131G>A causes uterine hyperplasia and leiomyomas in both *Med12* WT (*Med12^{Rmt/+} Amhr2-Cre*) and conditional KO (*Med12^{fl/+} Med12^{Rmt/+} Amhr2-Cre*) mice. *Med12* c.131G>A variant penetrance was 47% in mice on a WT background, while it reached 80% in mice on the conditional KO background. In mice on the conditional *Med12* deletion background, leiomyoma-like lesions tended to have earlier onset and achieve greater size. The *Med12* missense c.131G>A variant, therefore, acts as a gain-of-function mutation. *Med12* mouse mutations and genomic instability. Chromosomal rearrangements occur in 40% of human leiomyomas, and our data indicate that over 60% of uterine leiomyomas with an abnormal karyotype harbor *MED12* mutations (7). To assess the genomic profiles of the *Med12*-mutated mouse tumors, we conducted array comparative genomic hybridization (aCGH) on 4 uteri with leiomyoma-like lesions (Figure 1) and compared the profiles with those of uteri from littermate controls without Cre (*Med12^{fl/+} Med12^{Rmt/+}*). All 4 tumors showed genomic copy number gains and losses (40 per tumor), with mouse chromosomes 2, 7, 14, and 17 being most frequently affected. The affected regions often consisted of genes targeting cell cycle checkpoints or tumor pathways such as Ras, Wnt/ β -catenin, Tp53/Rb, NF- κ B, and TGF- β signaling. The complete list of aberrations in the uteri of *Med12^{fl/+} Med12^{Rmt/+} Amhr2-Cre* females is shown in Supplemental Table 1. Microarray analysis of *Med12^{fl/+} Med12^{Rmt/+}*

Table 1. *Med12^{fl/+} Med12^{Rmt/+} Amhr2-Cre* uteri chromosomal aberrations and corresponding human syntenic regions implicated in human leiomyomas

Chr	Gain/loss	Size (kb)	Genes of interest in region	Human syntenic loci
1qH5	Mosaic gain	104	<i>Rab3gap2</i> – TGF- β signaling; <i>lars2</i> – cell cycle checkpoint network; <i>Bpnt1</i> – estrogen metabolism	1q41
1qD	Mosaic loss	108	<i>Hjurf</i> – maintenance of genomic stability	2q37.1
4qD2.3	Mosaic loss	137	<i>Slc9A1</i> , <i>Map3k6</i> – MAPK/c-Jun signaling	1p36.1-p35
6qB1	Mosaic gain	105	<i>Prss1</i> – ECM receptors; <i>Prss3</i> – cell division	7q34
4qD2	Gain	40	<i>Adam28</i> – fibronectin receptor; <i>Adam 7</i> – collagen receptors	8p21.2
14qD3	Gain	133	<i>Pcdh17</i> – tumor suppression	13q21.1
7qA3.3	Mosaic gain	450	<i>Btbd9</i> – Tp53 network; <i>Glo1</i> – NF- κ B network; <i>Glp1r</i> – cAMP signaling	6p21.1-p21.3
18qA1	Mosaic gain	133	<i>Fzd8</i> – Wnt/ β -catenin network; <i>Ccny</i> – cell cycle regulator; <i>Cetn1</i> – chromosome segregation	10p11.21
18qA1	Loss	212	<i>Thoc1</i> – G2/M cell cycle checkpoint activator/apoptotic pathway	18p11.32

Chr, chromosome.

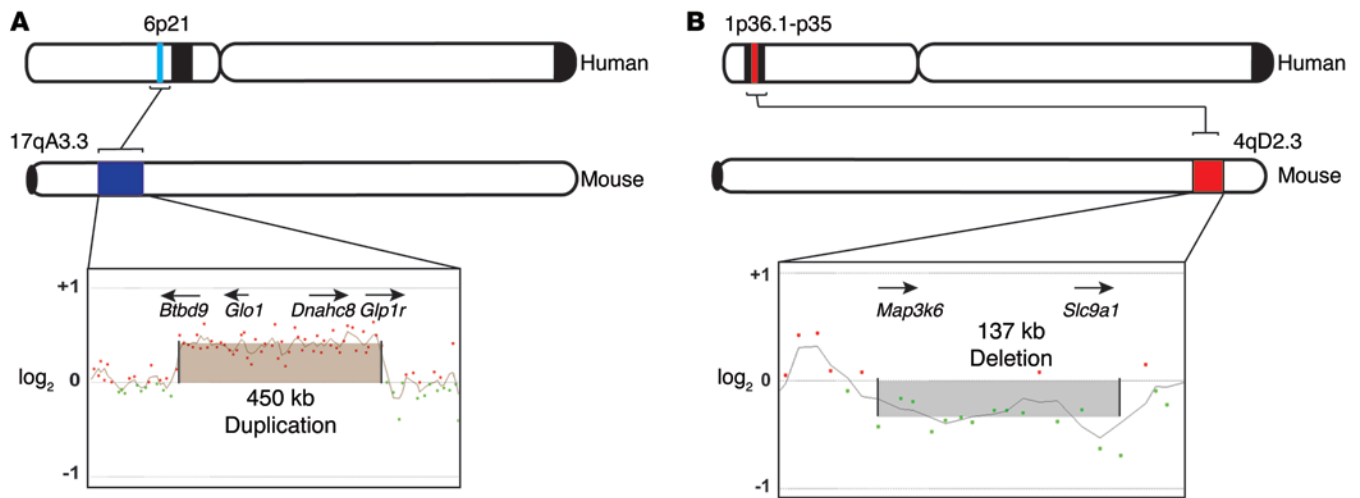


Figure 3. Representation of syntenic mapping of uterine rearrangements in *Med12^{fl/fl} Med12^{Rmt/+} Amhr2-Cre* female mice on human chromosomal loci. (A) Genomic duplication observed on mouse chromosomal locus 17qA3.3 is syntenic to the human 6p21 locus (shown in blue). A representative array profile of the 17qA3.3 region, highlighting the 450-kb duplication (chr17: 30586287–31049473), is also shown. (B) Genomic deletion observed on the mouse 4qD2.3 locus is syntenic to the human chromosomal locus 1p36.1–p35. The mouse deletion encompasses 137 kb and is shown in the respective array profile (chr4: 132799884–132936192). Positions are displayed approximately to scale according to the hg19 and mm9 physical maps, respectively.

Amhr2-Cre uteri also showed a few genomic regions with a pattern consistent with focal chromothripsis-like alterations (ref. 14 and Supplemental Figure 6A).

Approximately 50% of the mouse aberrations had syntenic counterparts on human chromosomes (Supplemental Table 2), and a number of these regions are known to be rearranged in human leiomyomas (Table 1). For example, mouse chromosome 17qA3.3, duplicated in *Med12^{fl/fl} Med12^{Rmt/+} Amhr2-Cre* uteri (Figure 3A), maps to the human 6p21 locus. Similarly, in another *Med12^{fl/fl} Med12^{Rmt/+} Amhr2-Cre* uterus, a deletion of the 4qD2.3 locus is syntenic to the human 1p36.1–p35 region (Figure 3B). Genomic rearrangements in 6p21 and 1p36.1–p35 are common in human leiomyomas. These results indicate that *Med12* mutations can cause the genomic instability frequently seen in human leiomyomas.

Recurrent human *MED12* exon 2 mutations have been associated with benign tumors such as uterine leiomyomas (7) and breast fibroadenomas (15); however, their etiology, genetic mechanism of action, and role in genomic instability are unknown. We generated mouse models with *Med12* loss of function and the most common human *MED12* c.131G>A variant and studied their effects in mouse uteri. Our mouse models show that the *Med12* c.131G>A variant alone drives tumor formation via a gain-of-function mechanism. These tumors histologically mimic human uterine leiomyomas, and their growth is affected by pregnancy, in accordance with human data.

There are 3 published Tg mouse models of uterine leiomyomas; these include Tg overexpression of hGPR10 driven with the calbindin-D9K promoter (16), conditional deletion of *Tsc2* (17), and conditional expression of a gain-of-function mutant form of β -catenin (18). The phenotype in these mice is confined to increased myometrial thickness and formation of small nodules, but none of these mice show the dramatic tumors we report here (Figure 1). Interestingly, *Amhr2-Cre* drives mutant *Med12* expression in both uterine smooth muscle (myometrium) and stroma, yet we only observed tumors derived from the mouse myometrium. In con-

trast, *Amhr2-Cre*-driven expression of the gain-of-function mutant form of β -catenin causes tumors in both the mouse myometrium and the stroma (18). These results indicate that *Med12* exon 2 mutations have specific tumorigenic effects in smooth muscle cells.

aCGH of *Med12* c.131G>A mouse uteri not only revealed genome-wide aberrations, but also showed complex chromosomal alterations such as chromothripsis. Recently, chromothripsis was reported in human leiomyomas and proposed as a possible mechanism of tumor progression (19). Chromosomal aberrations in mice also occur in regions that are syntenic to human 1p, 1q, 2q, 6p21, and 18p regions, also rearranged in human leiomyomas. It was previously shown that 60% of human leiomyomas with 6p21 rearrangements harbored *MED12* exon 2 mutations (20). These data suggest that *Med12* exon 2 mutations are precursors to genomic rearrangements and, hence, can cause genomic instability and drive tumor progression.

The limitations of our model include regulatory differences that may exist in the expression of *Med12* on the X chromosome versus on an autosome. Autosomal *Med12* is under the control of the *ROSA* promoter, which probably differs from the native *Med12* promoter. Nonetheless, our model mimics the human condition and shows that *Med12* variants can act through a gain-of-function mechanism. In the future, such models will provide a valuable tool for studying the role of *MED12* in the genesis of uterine leiomyomas and the specificity of its effects on smooth muscle cells.

Methods

Further information can be found in the Supplemental Methods.

Materials. *Med12^{fl/fl}* mice were a gift of Heinrich Schrewe (Max-Planck Institute, Berlin, Germany), and the *Amhr2-Cre* mice were a gift of Richard Behringer (The University of Texas, Houston, Texas, USA).

Statistics. A 2-tailed Student's *t* test was applied to determine the difference of means among groups using GraphPad Prism 4.0 (GraphPad Software). Statistical significance was defined at a *P* value of less than 0.05.

Study approval. All procedures were approved by the IACUC of the University of Pittsburgh and conducted in accordance with NIH guidelines for the care and use of laboratory animals.

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1. Stewart EA. Uterine fibroids. *Lancet*. 2001;357(9252):293–298.
2. Cramer SF, Patel A. The frequency of uterine leiomyomas. *Am J Clin Pathol*. 1990;94(4):435–438.
3. Sandberg AA. Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors: leiomyoma. *Cancer Genet Cytogenet*. 2005;158(1):1–26.
4. Nilbert M, Heim S, Mandahl N, Floderus UM, Willen H, Mitelman F. Characteristic chromosome abnormalities, including rearrangements of 6p, del(7q), +12, and t(12;14), in 44 uterine leiomyomas. *Hum Genet*. 1990;85(6):605–611.
5. Canevari RA, Pontes A, Rosa FE, Rainho CA, Rogatto SR. Independent clonal origin of multiple uterine leiomyomas that was determined by X chromosome inactivation and microsatellite analysis. *Am J Obstet Gynecol*. 2005;193(4):1395–1403.
6. Makinen N, et al. MED12, the mediator complex subunit 12 gene, is mutated at high frequency in uterine leiomyomas. *Science*. 2011;334(6053):252–255.
7. McGuire MM, Yatsenko A, Hoffner L, Jones M, Surti U, Rajkovic A. Whole exome sequencing in a random sample of North American women with leiomyomas identifies MED12 mutations in majority of uterine leiomyomas. *PLoS One*. 2012;7(3):e33251.
8. Bourbon HM. Comparative genomics supports a deep evolutionary origin for the large, four-module transcriptional mediator complex. *Nucleic Acids Res*. 2008;36(12):3993–4008.
9. Rocha PP, Scholze M, Bleiss W, Schrewe H. Med12 is essential for early mouse development and for canonical Wnt and Wnt/PCP signaling. *Development*. 2010;137(16):2723–2731.
10. Jamin SP, Arango NA, Mishina Y, Hanks MC, Behringer RR. Requirement of Bmpr1a for Mullerian duct regression during male sexual development. *Nat Genet*. 2002;32(3):408–410.
11. Wutz A. Gene silencing in X-chromosome inactivation: advances in understanding facultative heterochromatin formation. *Nat Rev Genet*. 2011;12(8):542–553.
12. Muzumdar MD, Tasic B, Miyamichi K, Li L, Luo L. A global double-fluorescent Cre reporter mouse. *Genesis*. 2007;45(9):593–605.
13. Bulun SE. Uterine fibroids. *N Engl J Med*. 2013;369(14):1344–1355.
14. Forment JV, Kaidi A, Jackson SP. Chromothripsis and cancer: causes and consequences of chromosome shattering. *Nat Rev Cancer*. 2012;12(10):663–670.
15. Lim WK, et al. Exome sequencing identifies highly recurrent MED12 somatic mutations in breast fibroadenoma. *Nat Genet*. 2014;46(8):877–880.
16. Varghese BV, et al. Loss of the repressor REST in uterine fibroids promotes aberrant G protein-coupled receptor 10 expression and activates mammalian target of rapamycin pathway. *Proc Natl Acad Sci U S A*. 2013;110(6):2187–2192.
17. Prizant H, et al. Uterine-specific loss of Tsc2 leads to myometrial tumors in both the uterus and lungs. *Mol Endocrinol*. 2013;27(9):1403–1414.
18. Tanwar PS, et al. Constitutive activation of Beta-catenin in uterine stroma and smooth muscle leads to the development of mesenchymal tumors in mice. *Biol Reprod*. 2009;81(3):545–552.
19. Mehine M, et al. Characterization of Uterine Leiomyomas by Whole-Genome Sequencing. *N Engl J Med*. 2013;369(1):43–53.
20. Markowski DN, Bartnitzke S, Loning T, Drieschner N, Helmke BM, Bullerdiek J. MED12 mutations in uterine fibroids-their relationship to cytogenetic subgroups. *Int J Cancer*. 2012;131(7):1528–1536.