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Clinical, Pathologic, Cytogenetic, and Molecular Profiling in Self-Identified Black Women with Uterine Leiomyomata

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Abstract

Black women are disproportionately affected by uterine leiomyomata (UL), or fibroids, compared to other racial groups, having a greater lifetime risk of developing UL and an earlier age of diagnosis. In order to elucidate molecular and genetic mechanisms responsible for the increased prevalence and morbidity associated with UL in black women, clinical, pathologic, cytogenetic, and select molecular profiling (*MED12* mutation analysis) of 75 self-reported black women undergoing surgical treatment for UL was performed. Our observations are broadly representative of previous cytogenetic studies of UL: karyotypically abnormal tumors were detected in 30.7% of women and 17.4% of analyzed tumors. No notable association was observed between race and increased occurrence of cytogenetic abnormalities that might contribute to any population-specific morbidity or prevalence rate. Our data on *MED12* mutation analyses (73.2% of tumors harbored a *MED12* mutation) provide additional support for a significant role of *MED12* in tumorigenesis. Although the effect of *MED12*-mediated tumorigenesis appears significant irrespective of race, other genetic events such as the distribution of karyotypic abnormalities appear differently in black

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women. This case series indicates that presently recognized genetic and molecular characteristics of UL do not appear to explain the increased prevalence and morbidity of UL in black women.

Graphical Abstracts/Highlights files

Common Cytogenetic Abnormalities Stratified by Chromosome

Chromosome Displaying Cytogenetic Abnormality	No. of Tumors Displaying Abnormality	Percent of All Abnormal Tumors (%)	Percent of All Tumors Analyzed (%)
chromosome 6	2	7.1	1.2
chromosome 7	15	53.6	9.3
chromosome 12	5	17.9	3.1
chromosome 14	0	0	0
other/complex	8	28.6	5.0

Heterozygous Point Mutations of MED12

Point Mutation	No. of Tumors with Mutation	Percent of All Point Mutations (%)	Percent of All Tumors Analyzed (%)
c.107T>G	3	12	7.5
c.128A>C	1	4	2.5
c.130G>A	2	8	5.0
c.130G>C	3	12	7.5
c.130G>T	3	12	7.5
c.131G>A	9	36	22.5
c.131G>T	4	16	10

Keywords

Fibroids; race; cytogenetic; molecular; clinical

INTRODUCTION

Uterine leiomyomata (UL), more commonly known as fibroids, are benign, clonal smooth muscle tumors of the uterus. UL are the most common pelvic tumor in women. By 50 years of age, 70% of white women and 80% of black women have had at least one fibroid. Severe symptoms, such as abdominal pain, abnormal menstrual bleeding, urinary incontinence, and fertility impairment, develop in 15–30% of these women during their reproductive years. [1] Uterine fibroids are a major public health concern given their high incidence, frequency, and morbidity, and are the primary indication for hysterectomy in the United States. [2–4] The annual direct cost for the clinical management of uterine fibroids in the United States is estimated to be $4.1–$9.4 billion, exclusive of costs attributable to obstetric complications

and lost productivity. [5] In addition, they contribute to a decreased quality of life for many women.

Black women are disproportionately affected by UL when compared to other racial groups. [6] In addition to having a greater lifetime cumulative incidence of fibroids, black women are also diagnosed at a younger age. Uterine fibroids are significantly larger at the time of diagnosis in black women compared to white women and are associated with longer phases of sustained growth. [1] Affected black women are also more likely to have multiple fibroids. [7] Even after controlling for known risk factors such as BMI and hypertension, race remains a factor predisposing black women to develop UL, supporting an underlying genetic contribution. [8] In addition to this racial difference in prevalence and morbidity, a genetic component to UL predisposition is substantiated by analyses of twin studies and familial aggregation. Further, cytogenetic and molecular studies have provided evidence of a strong genetic component in the pathobiology of these tumors. [9–10]

Approximately 40% of uterine fibroids are chromosomally abnormal. Consistent, non-random cytogenetic abnormalities account for the majority of these aberrations, notably deletions of 7q, trisomy 12, or rearrangements of 12q15, 6p21, or 10q22. Additional chromosomal abnormalities of varying complexity have been routinely identified. [11–12] Three independent genetic subtypes of UL have emerged with the advent of next-generation sequencing technologies: rearrangements of the gene encoding high-mobility group protein AT-hook 2 (*HMGA2*); mutations of fumarate hydratase (*FH*); and mutations of the mediator complex subunit 12 gene (*MED12*). [11,13–14] *HMGA2* is dysregulated in UL with chromosomal rearrangements of 12q15. [15] While mutations of *FH* at 1q43 are known to encode syndromic forms of UL such as multiple cutaneous and uterine leiomyomata (MCL) and hereditary leiomyomatosis and renal cell cancer (HLRCC), loss of *FH* may also play a role in the pathogenesis of nonsyndromic UL. [11] Mutations in exon 2 of *MED12* have been reported in 50%–70% of UL. [16]

A thorough understanding, however, of molecular and genetic mechanisms responsible for the increased prevalence and morbidity associated with UL among black women is needed to inform future research directions and clinical treatments. The aim of this case series is to present clinical, pathologic, cytogenetic, and select molecular profiles (*MED12* mutation analysis) of 75 self-reported black women with UL undergoing surgical treatment at Brigham and Women's Hospital who enrolled in our ongoing UL-related research studies over a 27-year period.

MATERIALS AND METHODS

Human Subjects Study Approval

Approval for this study was obtained from the Partners Human Research Committee/ Institutional Review Board of Partners Healthcare System (Boston, MA).

Study Population

The Center for Uterine Fibroids at Brigham and Women's Hospital (Boston, MA; www.fibroids.net) has had a longstanding interest in understanding the genetic

underpinnings of UL. Women undergoing surgery in the Department of Obstetrics and Gynecology for treatment of UL are consented and enrolled in our research studies. Self-identified black women presented in this report underwent either a myomectomy or hysterectomy at Brigham and Women's Hospital between 1989 and 2015.

Medical Record Review

Clinical records of all subjects were reviewed. Information such as patient's age at time of treatment, clinical indication or primary symptom for treatment, tumor size and number, and uterine weight were analyzed.

Tissue Handling and Cytogenetic Analysis

Samples of UL and matched myometrium were collected during or immediately following surgery. For subjects with multiple fibroids, the largest fibroids were selected for analysis. In instances where a minimally invasive morcellation procedure was performed and delineation of individual fibroids was not possible, only a single piece of tissue was selected for study. Tissue for DNA isolation was frozen and stored at −80°C. Tissue for cell culture and cytogenetic analysis was transferred to Hank's balanced salt solution. From tissue samples collected in Hank's, cell cultures were established as previously described, and standard GTG-banded karyotyping was performed. [12] Formalin-fixed, paraffin-embedded tissue blocks were obtained for histopathologic analysis and confirmation of UL diagnosis.

DNA Isolation and MED12 Mutation Analysis

DNA was isolated from frozen tissue samples using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Using previously reported primer sequences, the desired DNA fragment, exon 2 of *MED12*, was amplified with Invitrogen Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA). [16] Subsequently, PCR products were separated by agarose gel electrophoresis. DNA fragments were extracted using the Qiagen Gel Extraction Kit. DNA sequencing was then performed on an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Waltham, MA) using forward and reverse PCR primers. Sequence chromatographs were analyzed using Geospiza's FinchTV software (Geospiza Inc., Seattle, WA).

RESULTS

Clinical Evaluation

Our study group consisted of 75 self-reported black women with a confirmed diagnosis of UL. All patients with symptomatic uterine fibroids and a supporting physical examination underwent further ultrasonographic evaluation to confirm UL diagnosis as well as to define the location, size and number of fibroids. Mean age at the time of surgical treatment was 39.5 years (median 39, range 28–57). Forty-one women underwent a myomectomy (36 abdominal myomectomies, five laparoscopic myomectomies), and 34 underwent a hysterectomy (six supracervical hysterectomies, 27 total abdominal hysterectomies, one total vaginal hysterectomy). Fourteen women undergoing hysterectomy and two undergoing myomectomy had either a concurrent unilateral or bilateral salpingo-oophorectomy. Of note, our data on surgical procedures are demonstrative of broader trends in surgical management

of UL over the past few decades toward more minimally invasive and uterine-conserving procedures. [17]

The primary indication or symptom prompting medical or surgical intervention was abnormally heavy or prolonged menstrual bleeding, or menorrhagia, with 40 women (53.3%) reporting this symptom. Twenty-eight women (37.3%) reported pelvic pain or pressure. Twelve women (16%) were undergoing surgical intervention in response to fertility complications. Ten women (7.5%) reported urinary impairment such as incontinence. Other indications included abdominal pain, increased abdominal girth, or a pelvic/abdominal mass. Twenty-one women (28%) reported two or more of these symptoms.

Clinical management of UL frequently involves treatment with gonadotropin-releasing hormone agonists (GnRHa) to provide relief from excessive vaginal bleeding and to reduce uterine volume and fibroid size prior to surgery. [18] Sixteen women (21.3%) had received leuprolide, a GnRHa, prior to surgical intervention.

Hysterectomy remains the only essentially curative treatment for UL. Between 10% and 25% of women who have a myomectomy will require additional surgical intervention for recurrent fibroids. [19] In our study, 13 women (17.3%) had undergone previous surgical intervention before their procedure at Brigham and Women's Hospital. The average time since most recent surgical intervention for UL was 44.1 months (median 48, range 3.5–84) across the cohort. Eleven women (14.7%) who underwent a myomectomy at the time of the study required future surgical intervention. The average time until subsequent intervention was 74.5 months (median 62.8, range 1.63–168). Five women who had undergone a previous surgical intervention opted for a hysterectomy. Of note, one woman underwent three hysteroscopic myomectomies before undergoing a total abdominal hysterectomy with bilateral salpingo-oophorectomy as part of a treatment plan for uterine adenosarcoma. Fully comprehensive follow-up was not possible for all subjects given that our access to medical record was restricted to only those women who continued their care in the Partners Healthcare System network.

Pathologic Evaluation

For women undergoing hysterectomy, the mean uterine weight was 591.9 grams (median 401, range 66–4678). The mean uterine diameters were 12.8 × 10.1 × 7.3 centimeters. Across the entire cohort, the mean number of tumors per woman indicated by gross pathologic examination was 18.2 (median 11, range 1–109). The exact number of tumors present for 29 women was unobtainable due to either absent information or a record only of "multiple" fibroids. The mean value of the greatest tumor dimension was 7.8 centimeters (median 6.5, range 0.5–17). Reported tumor locations were subserosal (51 women), intramural (49), submucosal (31), and fundal (15). Fourteen women had pedunculated fibroids. Fifty-three women had tumors in two or more locations. Twenty-nine women had tumors in three or more locations.

A more thorough histopathologic analysis was performed for 40 cases. Thirty-three cases were diagnosed as usual type UL, two as usual type UL with hyalinization, one as usual type UL with myxoid changes, one as usual type UL with plexiform changes, and three as

cellular/focally cellular UL. Additionally, pathologic evaluation of the tumors indicated 13 cases of adenomyosis. Twenty-eight cases had accompanying chronic cervicitis, cervical squamous metaplasia, mucous cysts, or parakeratosis.

Cytogenetic Evaluation

One hundred sixty-one individual tumors (an average of ~two tumors per case) were successfully karyotyped. Twenty-eight of these tumors (17.4% of all tumors analyzed) from 23 cases (30.7%) were karyotypically abnormal. In the 47 cases in which more than one tumor was successfully karyotyped, 15 cases (31.9%) had tumors with both abnormal and normal karyotypes. Of the 16 subjects reported to have received leuprolide treatment, eight subjects (50%) had a cytogenetic abnormality identified in their tumors. Table 1 provides information on all tumors which had an abnormal karyotype or a recognized constitutional variant. Abnormalities are arranged by the most common cytogenetic rearrangements associated with UL in Table 2. [20] The most frequent cytogenetic abnormality observed was an interstitial deletion in the long arm of chromosome 7.

Molecular Evaluation

Forty karyotyped tumors from 28 cases were analyzed for the presence of mutations in exon 2 of *MED12*. Selection of a tumor for *MED12* mutation analysis was based solely on availability of tissue. Mutations were found in 30 (75%) of tumors. The most common mutations were heterozygous point mutations in codons 43 and 44 (25 tumors, 62.5% of tumors analyzed). There were also five cases with deletions spanning exon 2. Tumors from five subjects who received leuprolide treatment were analyzed for presence of a *MED12* mutation; three subjects had tumors containing *MED12* mutations (60%) occurring exclusively in the coding region of exon 2. Additionally, Mäkinen et al. [16] performed whole exome sequencing and reported mutations only in exon 2, and no mutations in noncoding regions adjacent to *MED12*. Only two tumors had rearrangements involving 12q15, one of which had a *MED12* mutation. Detailed information on *MED12* analysis is provided in Table 3, and the distribution of the different *MED12* point mutations is shown in Table 4.

DISCUSSION

The impact of ancestral origin in the biology of uterine leiomyoma is not well understood, although it is well recognized as a risk factor. In regard to genetic studies, large-scale genome-wide association studies and exome sequencing analyses, efforts, and discoveries have been focused on populations of European and Asian ancestry. [10,16,21] There is evidence, however, of racial differences for fibroid development on a molecular level, a discovery that may help guide treatment. [22–24] The information presented in this case series seeks to contribute to a greater understanding of fibroid development on a genetic and molecular level in a disproportionately affected population. In 2013, Moorman et al. [25] published a study on the pathologic and epidemiologic risk factors for UL in black and white women undergoing pre-menopausal hysterectomy. Their analysis of 225 black and 135 white women yielded similar results to our study. However, the study described herein finds a greater mean number of fibroids per patient (18.2 versus 9.9) as well as a greater uterine

weight (591.9 g versus 477). [25] A 1996 study by Kjerulff et al. [26] with 409 black women reported a mean uterine weight of 420.8 g. A possible explanation for a seemingly more severe clinical presentation in our study group may be due to the nature of the gynecologic surgical practice at Brigham and Women's Hospital being primarily referral based and, therefore, a selection bias toward more severe and complex clinical presentations.

Limited published data exist on the cytogenetic abnormalities associated with fibroids specifically in black women. Multiple separate analyses, using predominantly white or non-population-specific cohorts, have demonstrated that approximately 40% of UL tumors are cytogenetically abnormal. [27–29] Thirty-one percent of women in our study had fibroids with cytogenetic abnormalities, while 17.4% of all analyzed tumors were cytogenetically abnormal. Therefore, our cohort broadly resembles previous UL cytogenetic studies with regard to rate of cytogenetic abnormality. More specifically, our data are not supportive of an association between an increased rate of cytogenetic abnormality and race that may contribute to any population-specific morbidity or prevalence. However, one of the most common cytogenetic rearrangements associated with UL, t(12;14), typically found in 20% of all chromosomally abnormal cases was present in only three cases (10.7% of abnormal tumors). Of interest, the distribution of numbers of CT repeats in the 5′ UTR of *HMGA2* at 12q15 is strikingly different in black and white women. [30–31] The number of CT repeats (n=27) has been associated with a predisposition to develop UL in a study of white women, and also associated with short stature in this study. [31] Although it remains to be proven, the presence of the 27 CT repeats may potentially mediate t(12;14) and underlie the differential distributions of t(12;14) in black and white women. [31] An enrichment of tumors with deletions of segments of chromosome 7 was observed with 53.6% of all abnormal tumors harboring this aberration. This deletion is reportedly found in 17% of all chromosomally abnormal cases. [32] The other types of abnormalities observed in our cohort are largely consistent with previous studies. [11, 29] However, six tumors (21.4% of abnormal tumors) were observed with very complex cytogenetic abnormalities, and may indicate regions of the genome harboring other genes with pathogenetic variants in the biology of UL.

Since Mäkinen et al. [16] published on the presence of various somatic mutations in exon 2 of *MED12* in 2011, numerous mutation analysis studies have been performed in a variety of study populations. [33–38] Mäkinen et al. [39] also published on a separate cohort of 18 women (28 UL) from South Africa identified as "black South African" or "coloured" in 2013. Their mutation rate of 50% of tumors (versus 75% in our group) suggests that *MED12* mutations are not substantially more common in black women given that the suggested prevalence of *MED12* mutation has ranged from 50% to 70%. [39] Our data provide supportive evidence of a significant role of *MED12* in tumorigenesis, irrespective of race or ethnicity.

When our data on *MED12* mutations are analyzed in the context of cytogenetic abnormalities, particularly t(12;14), and vice versa, no clear pattern or trend emerges. Of note, however, is the mosaicism among tumors in regard to *MED12* mutations belonging to the same case. In 2012, Markowski et al. [20] stratified UL *MED12* mutations by cytogenetic subgroup in a German population of 50 patients (80 UL). Our comparatively

modest sample size of 28 patients (40 UL) analyzed for *MED12* mutation makes any strongly substantiated claims difficult. However, the results from our cohort largely track those of Markowski et al. [20] who reported that 21.9% of their cytogenetically abnormal and 82.6% of their cytogenetically normal tumors displayed *MED12* mutations. Of the nine cytogenetically abnormal tumors in our cohort, 66.7% displayed *MED12* mutations, and 77.4% of the cytogenetically normal tumors analyzed displayed *MED12* mutations.

MED12 is a 45-exon gene on chromosome Xq13 that encodes a single subunit of the Mediator multiprotein complex. [40] Mediator plays critical regulatory roles in multiple, global transcriptional processes, including assembly, initiation, and elongation of the RNA polymerase II at and across gene bodies. *MED12* regulates activation of CDK8 kinase by bridging the protein-protein interaction between Cyclin C-CDK8 to the core of the Mediator complex. This interaction acts as a functional switch for alternating between inclusion and exclusion of Cyclin C-CDK8 in the body of the Mediator multiprotein complex, as it corresponds to the subcomplex transitioning between transcriptionally active and repressive states. [40–49] Mutations in exon 2 of *MED12* appear to disrupt critical interactions between Cyclin C and CDK8, possibly dysregulating the Mediator complex altogether in a high frequency (~70%) of (UL). [20,50] High frequency, driver mutations located in exon 2 of *MED12* are hypothesized to decrease Mediator-associated CDK8 activity at a functional regulatory switch, causing alterations in the global transcriptional programs of UL. [14, 50]

Additional genomic analyses are warranted to determine the landscape that differentiates this gynecologic disorder in women of different ethnic groups. Specifically, advances in next generation sequencing technologies, such as exome and whole genome sequencing, as well as SNP arrays can be used to identify somatic copy number variations in UL tumors. [51–52] Copy number variation arrays can also be used to identify small genomic imbalances and chromothripsis in UL tumors. [53–55] Furthermore, the inclusion of more comprehensively profiled cases of UL in black women is necessary to add sufficient power and significance to our findings. Nonetheless, this case series demonstrates that currently identified genetic and molecular characteristics of UL do not apparently account for the increased prevalence and morbidity of UL in black women.

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Study data were collected and managed using REDCap electronic data capture tools hosted at Partners Healthcare. [56]

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Table 1

Cytogenetic Abnormalities Observed in Uterine Leiomyomata

Subject	Age at Diagnosis (years)	No. of Fibroids	Case Number	Karyotype	Cytogenetic Abnormality	*MED12* Mutation (if analyzed)
UL02	48	multiple	ST91-328	43,X,-X,del(1)(p32->pter),−6,del(7)(q22q32), −19[16]/46,XX[7]	complex	
UL03	46	12	ST91-314	46,X,r(X)[8]/46,XX[13]	ring	
			ST91-315	46,XX		
UL07	46	5	ST93-248 *	46,XX,inv(9)		
			ST93-249 *	46,XX,inv(9)		
UL09	49	multiple	ST99-045	46,XX,del(7)(q22q32)[8]/46,XX[2]	del(7)	
			ST99-046	46,XX,del(7)(?q11?q11.2)[2]/46,XX[4]	del(7)	
			ST99-048	46,XX		
			ST99-049	46,XX		
UL15	38	6	ST89-185	46,XX		
			ST89-186	50,XX,dup(12)(q14->q24),+21,+21,+21,+21[1]/46,XX[65]	complex	
UL16	38	multiple	ST99-735	46,XX		
			ST99-736	46,XX		c.107T>G
			ST99-737	46,XX,t(6;10)(p21;q22)	6p rea	c.130G>A
			ST99-738	46,XX		
UL19	40	19	ST93-470	46,XX,del(7)(q22q32)[19]/46,XX[1]	del(7)	
			ST93-471	46,XX		
UL21	46	multiple	ST99-122	45,XX,−22[6]/46,XX[2]	monosomy 22	
			ST99-123	46,XX,del(7)(q22q32)[11]	del(7)	
UL23	40	multiple	ST94-190 *	46,XX,9qh+		
			ST94-191 *	46,XX,9qh+		
			ST94-192 *	46,XX,9qh+		

Subject	Age at Diagnosis (years)	No. of Fibroids	Case Number	Karyotype	Cytogenetic Abnormality	*MED12* Mutation (if analyzed)
			ST94-193	46,XX,del(7)(q21.1q34),9qh+[3]/46,XX,9qh+[17]	del(7)	
UL25	38	3	ST93-054	46,XX		
			ST93-055	45,XX,t(1;7)(q31;q22),−2,−3,−6,add(11)(q21),−18,+mar1,+mar2,+mar3	complex	
			ST93-056	46,XX		
UL26	39	21	ST95-479	46,XX,del(7)(q22q32)[6]/46,XX,?inv(7)(q21q22)[2]/46,XX[10]	del(7)	
			ST95-480	46,XX		
UL27	38	multiple	ST95-520 *	46,XX,inv(9)		
			ST95-521 *	46,XX,inv(9)		
			ST95-522 *	46,XX,inv(9)		
UL32	38	5	ST96-437	46,XX,del(7)(q22q32)[1]/46,XX[14]	del(7)	
UL37	36	multiple	ST95-364	46,XX		
			ST95-365	46,XX,t(1;13)(p22;q14),−11,+mar?(11pter- >q12:)[8]/46,XX[2]	complex	
			ST95-366	46,XX		
UL40	33	5	ST93-731	46,XX		
			ST93-732	46,XX		
			ST93-733	46,XX,del(7)(q22q32)[13]/46,XX[2]	del(7)	
UL41	39	multiple	ST00-025	45,XX,r(?13),−22	ring	
			ST00-026	46,XX		c.107T>G
UL42	42	multiple	ST02-278	46,XX,t(10;12)(q23;q15)	t(10;12)	c.131G>A
UL48	33	multiple	ST96-481	46,XX		
			ST96-482	46,XX,del(7)(q22q32)[10]/46,XX[10]	del(7)	
UL51	43	14	ST09-009	46,XX		
			ST09-010	45,X,-X,t(6;16)(q13;q24),del(7)(q22)	del(7)	
			ST09-011	46,XX		c.116-154del39

Subject	Age at Diagnosis (years)	No. of Fibroids	Case Number	Karyotype	Cytogenetic Abnormality	*MED12* Mutation (if analyzed)
			ST09-012	46,XX		
UL53	40	3	ST08-014	46,XX,del(7)(q22q23)	del(7)	c.122_156del35
			ST08-015	46,XX,t(1;6)(p36;p21)[8]/46,XX[13]	t(1;6)	c.130G>C
UL54	34	multiple	ST01-901	45,X,-X,r(1),t(4;6)(p16;q21),der(12)inv(12)(p13q11)ins(12)t(12;14)(q15;q21q24),del(14)(q21q24)	complex	wt
UL56	39	15	ST08-021	46,XX		
			ST08-022	46,XX		
			ST08-023	46,XX,del(7)(q11q31)	del(7)	c.131G>A
UL57	34	11	ST05-001F1 *	46,XX,9qh+,22pstk+		
			ST05-001F2 *	46,XX,9qh+,22pstk+,+mar[3]/46,XX,9qh+,22pstk+[7]		
UL61	37	9	ST09-007	47,XX,+12	trisomy 12	wt
UL62	35	13	ST08-017 *	46,XX,inv(19)		c.131G>A
			ST08-018 *	46,XX,inv(19)		wt
			ST08-019 *	46,XX,inv(19)		wt
UL64	29	2	ST02-919	46,XX,t(1;14;12)(q21;q24;q15)	t(1;14;12)	
			ST02-920	47,XX,+add(1)(p13),add(12)(q15)	complex	
UL69	29	3	ST09-036	46,XX		c.131G>A
			ST09-037	46,XX,+12	trisomy 12	
			ST09-038	46,XX,del(7)	del(7)	

* inv(9), 9qh+, inv(19) and 22pstk+ interpreted as constitutional variants not of clinical significance

Table 2

Common Cytogenetic Abnormalities Stratified by Chromosome

Chromosome Displaying Cytogenetic Abnormality	No. of Tumors Displaying Abnormality	Percent of All Abnormal Tumors (%)	Percent of All Tumors Analyzed (%)
chromosome 6	2	7.1	1.2
chromosome 7	15	53.6	9.3
chromosome 12	5	17.9	3.1
chromosome 14	0	0	0
other/complex	8	28.6	5.0

Table 3

MED12 Mutation Analysis

Subject	Age at Diagnosis (years)	No. of Fibroids	Greatest Fibroid Dimension (cm)	Case Number	*MED12* Mutation	Karyotype
UL16	38	multiple	6.5	ST99-736 ST99-737	c.107T>G, p.L36R c.130G>A, p.G44S	46,XX 6p rea
UL36	42	multiple	12.5	ST00-399	c.IVS1-9_140del50	46,XX
UL38	42	24	4.5	ST02-020 ST02-021	c.107T>G, p.L36R c.130G>T, p.G44C	46,XX 46,XX
UL41	39	multiple	5.8	ST00-026	c.107T>G, p.L36R	46,XX
UL42	42	multiple	12	ST02-278	c.131G>A, p.G44D	t(10;12)
UL46	50	33	7.5	ST11-002T2	c.130G>C, p.G44R	46,XX
UL50	45	8	12.5	ST09-029	c.130G>T, p.G44C	46,XX
UL51	43	14	15	ST09-009 ST09-010 ST09-011	c.143_168del26, p.Q48_H56 c. 31G>A, p.G44D c.116-154del38, p.L39_V51	46,XX del(7) * 46,XX
UL52	44	19	5	ST09-024	c.130G>T, p.G44C	46,XX
UL53	40	3	9.5	ST08-014 ST08-015	c.122_156del35, p.V41_S52 c.130G>C, p.G44R	del(7) t(1;6)
UL54	34	multiple	10	ST01-901	wt	complex
UL55	33	28	8	ST02-137 ST02-138	c.130G>A, p.G44S c.130G>C, p.G44R	46,XX 46,XX
UL56	39	15	9	ST08-023	c.131G>A, p.G44D	del(7)
UL57	34	11	10	ST05-001F1	c.131G>A, p.G44D	46,XX,9qh+,22pstk+ **
UL58	37	3	5	ST08-025	c.131G>T, p.G44V	46,XX

Subject	Age at Diagnosis (years)	No. of Fibroids	Greatest Fibroid Dimension (cm)	Case Number	*MED12* Mutation	Karyotype
UL60	40	6	8	ST12-006T1	c.131G>T, p.G44V	46,XX
				ST12-006T2	wt	46,XX
UL61	37	9	5	ST09-007	wt	trisomy 12
UL62	35	13	17	ST08-017	c.131G>A, p.G44D	inv(19) **
				ST08-019	wt	inv(19) **
UL63	35	9	10	ST09-002	wt	46,XX
UL64	29	2	12.5	ST02-919	wt	t(1;14;12)
UL65	35	9	7.8	ST09-020	wt	46,XX
UL66	38	41	15	ST12-011T1	c.128A>C, p.Q43P	46,XX
				ST12-011T2	c.90_101del12, p.F30_D34	46,XX
UL67	35	15	10.5	ST12-002T1	c.131G>T, p.G44V	46,XX
				ST12-002T2	c.131G>A, p.G44D	46,XX
UL68	33	13	12	ST13-001T2	c.131G>T, p.G44V	46,XX
				ST13-001T3	wt	46,XX
UL69	29	3	12	ST09-036	c.131G>A, p.G44D	46,XX
UL70	30	105	16.5	ST12-001T1	wt	46,XX
UL71	28	7	12	ST12-003T1	c.131G>A, p.G44D	46,XX
UL73	35	11	3.5	ST11-001T1	c.131G>A, p.G44D	46,XX
				ST11-001T2	wt	46,XX

* del(7) indicates the characteristic interstitial deletions seen in UL in the long arm of chromosome 7 with various breakpoints

** inv(9), 9qh+, inv(19), 22pstk+ interpreted as constitutional variants not of clinical significance

Table 4

Heterozygous Point Mutations of MED12

Point Mutation	No. of Tumors with Mutation	Percent of All Point Mutations (%)	Percent of All Tumors Analyzed (%)
c.107T>G	3	12	7.5
c.128A>C	1	4	2.5
c.130G>A	2	8	5.0
c.130G>C	3	12	7.5
c.130G>T	3	12	7.5
c.131G>A	9	36	22.5
c.131G>T	4	16	10