Cancer Genetics 222-223 (2018) 1–8

Cancer Genetics

ORIGINAL ARTICLE

# Clinical, pathologic, cytogenetic, and molecular profiling in self-identified black women with uterine leiomyomata

Mark A. Hayden [a], Zehra Ordulu [a,b], C. Scott Gallagher [b,c], Bradley J. Quade [b,d], Raymond M. Anchan [a,b], Nia Robinson Middleton [a,b], Serene S. Srouji [a,b], Elizabeth A. Stewart [e], Cynthia C. Morton [a,b,d,f,g,*]

[a] *Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Boston, MA 02115, USA;* [b] *Harvard Medical School, Boston, MA 02115, USA;* [c] *Department of Genetics, Harvard Medical School, Boston, MA 02115, USA;* [d] *Department of Pathology, Brigham and Women's Hospital, Boston, MA 02115, USA;* [e] *Department of Obstetrics and Gynecology, Mayo Clinic, Rochester, MN 55905, USA;* [f] *Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA 02142, USA;* [g] *University of Manchester, Manchester Academic Health Science Centre, Manchester, UK*

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 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.

**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

This study was conducted in Boston, MA.

Received September 4, 2017; received in revised form January 8, 2018; accepted January 15, 2018.

* Corresponding author.
*E-mail address:* cmorton@bwh.harvard.edu

2210-7762/$ - see front matter © 2018 Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.cancergen.2018.01.001

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