Leiomyomata Uteri: Hormonal and Molecular Determinants of Growth

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Objective: To review the available English literature that examines the biology of leiomyoma uteri in African-American women and other ethnic groups. Factors that influence the growth and development of leiomyomas are examined to understand the basis for larger myomas in African-American women.

Design: Literature review of 176 articles regarding the pathobiology of leiomyoma in various ethnic groups.

Results: The initiating factor(s) associated with the transformation of a normal myometrial cell into a leiomyoma cell remain(s) to be determined. Epidemiological studies have confirmed that different ethnic groups develop leiomyomas. However, African-American ethnicity is a risk factor for the development of leiomyomas. Studies have examined diet, genetics, hormonal, growth, enzymatic and molecular determinants of myoma biology, with critical advances in some of these areas. The best radiological tools to identify and monitor leiomyomas are ultrasonography and/or magnetic resonance imaging. Evidence supports progesterone and growth factors (e.g., transforming growth factor-ß), have significant impact on the development of leiomyomas.

Conclusions: Early monitoring and intervention should become standard for African-American women who are at greater risk for developing leiomyomas. There are plausible biological mechanisms that explain the predisposition for developing larger leiomyomas in African-American women as compared with other ethnic groups.

Key words: African Americans ■ women's health ■ leiomyoma ■ myoma ■ pathobiology

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Leiomyomata uteri, commonly called uterine fibroids (fibroids), are the most common benign neoplasia that develop within the muscular layer of the uterus in women. They are typically asymptomatic, but up to 30% of American women experience clinical symptoms. Generally, women present two types of complaints—abnormal uterine bleeding and/or lower-abdomen pressure-related symptoms. Various terminologies are used to characterize this uterine tumor: myofibroma, fibromyoma, leiomyofibroma, leiomyoma, fibroma, myoma and fibroid. The designation "fibroid" is the least accurate. Nonetheless, it is the most commonly used diagnostic term in both scientific and lay literature. Leiomyoma accurately describes this neoplasm and refers to any benign tumor of smooth-muscle origin.

The term "leiomyoma" captures the predominant components of these tumors as well as the cell of origin. It is unknown whether a leiomyoma actually transforms into a malignant phenotype termed "leiomyosarcoma." However, the incidence of leiomyosarcoma is extremely low in premenopausal patients when compared to older postmenopausal women, wherein they account for <1% of uterine malignancies. Apart from their tumorigenic potential, they are morphologically similar at the cellular level to normal myometrial smooth-muscle cells (MSMCs). Leiomyomas may have single or multiple mutated smooth-muscle tumor nodules of varying size attached and/or within the myometrium that are encircled by varying amounts of extracellular fibrous connective tissue. Briefly, the histopathology reveals that they are well circumscribed, pseudoencapsulated, solid and pearly white or lightly tanned round masses with size ranging from 1 mm–>30 cm (Figure 1) simulating a pregnant uterus. Microscopic determinations reveal they have interlacing bundles of spindle-shaped or stellate smooth-muscle cells with little cellular pleomorphism or mitotic activity (<5/10 hpf). It is interesting to note that their growth is associated with low mitotic activity.

Although leiomyomata are prevalent within the uterus, they may develop elsewhere in the body, e.g., gastrointestinal tract or within the walls of arteries—intravenous leiomyomatosis. Within a normal uterus (Figure 2), they can grow entirely within the myometrial compartment (intramural or interstitial leiomyomas, Figure 3), protrude through the serosal surface of the uterus into the peritoneal cavity (pedunculated and subserosal

leiomyomas, Figure 4) or project into the uterine cavity (submucosal leiomyoma, Figure 5). There are fewer occurrences of leiomyomata detected in the cervix and in the fallopian tubes. It is also uncommon for the leiomyomata to grow into the broad ligament (Figures 6 and 7) and acquire blood supply from other intraperitoneal organs (parasitic leiomyoma).

Leiomyomata uteri are common during the reproductive years, and several reports by Thompson ISI (www.isinet.com) indicate they have a widespread geographic distribution. Thompson's database spans 11 years (1994 to December 31, 2004), includes 80 countries and has reports in >700 different journals with >954 articles on leiomyomata. Although no firm statistical determination can be made at this time, Thompson's report supports the conclusion that leiomyomata uteri are ubiquitous.

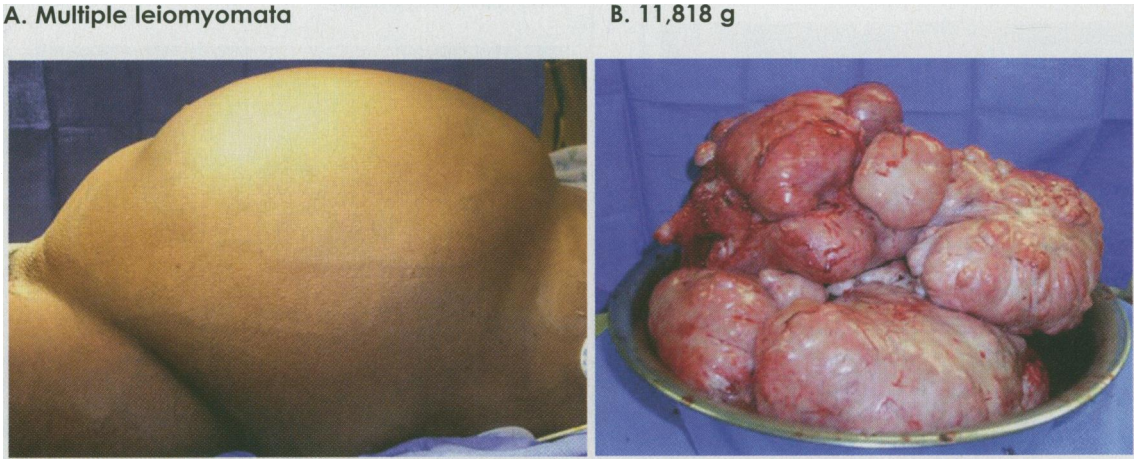
While it is clear that leiomyoma uteri have been diagnosed in several ethnic groups, only African-American women are at greater risk for developing these tumors. Because of this apparent predisposition for developing leiomyoma, it has become widely recognized in literature that premenopausal African-American women experience an increased prevalence of the clinical symptoms attributed to both the size and number of leiomyomas encountered at surgical management. This review reports and examines key hormonal and molecular determinants of leiomyoma pathobiology that influence their development.

ETIOLOGY, CYTOGENETICS AND NATURAL HISTORY

Miller and Ludovici[1] were the first to determine that the smooth-muscle cell of the uterus is the origin of these benign uterine neoplasms. They acquire varying size through cellular proliferation and altered apoptosis. The exact event(s) that transform(s) normal MSMCs into leiomyoma cells remains to be determined. Several theories[2-7] have been advanced attempting to characterize the mechanism(s) leading to myoma formation. Flake et al.[8] have reviewed some of these theories.

Considerations regarding etiology include a diverse set of environmental compounds that may originate from industrial, pharmaceutical or dietary sources. These compounds are known as xenoestrogens. They exist as natural, e.g., genistein (natural isoflavone found in soybeans) or synthetic compounds, e.g., diethylstilbestrol (DES). They have varying chemical structures that can bind to estrogen receptor (ER) in the myometrium and have the potential for either estrogen agonist or antagonistic effects. Although, these compounds have the potential to alter some aspect of the cellular functions of MSMCs, none of the xenoestrogens have been specifically linked in the transformation of normal MSMCs into myomas in humans.[8] It is clear, however, that somatic mutations and/or less frequent molecular alterations in the X chromosome within the MSMCs must occur for the initiation and subsequent development of a myoma. The high prevalence of leiomyomata suggests that they result from stable mutation(s). Cytogenetic studies of leiomyoma cells (leiomyocyte) are designed to locate the precise type(s) of chromosomal disruption present and the accompanying genes linked to leiomyoma formation and/or growth. Mutations are not always evident from these analyses. The cytogenetic studies of different leiomyomata reveal that 40% have nonrandom tumor-specific chromosomal abnormalities. The other 60% of leiomyomata have normal chromosomal profiles.[9,10] Conventional dogma declares that the presence of normal karyotypes does not preclude submicroscopic genetic aberration(s). Thus, the precise initiator(s) of genomic change(s) in MSMC remain(s) to be determined. However, we can safely assume from the prevalence of this condition that the following is probable: the early mutated MSMCs must have selective growth advantages allowing for the proliferation of the mutated cells and their potential responsivity to growth pro-

Figure 1.



moting extracellular and intracellular factors. The tenet that these tumors grow by clonal expansion from a single progenitor cell in which the genetic abnormality occurred has been challenged.[11-13] These authors[11,12] suggest that chromosomal rearrangements develop as a secondary event during the monoclonal expansion of an existing leiomyoma.

This observation does not alter the fact that there are specific and reoccurring chromosomal rearrangements that involve genes known to be critical to the development of leiomyomata.[14,15] Coincident with the thesis that unknown factor(s) are essential to initiate transition from a normal myocyte into a myoma is the observation that none of the current hypotheses[2-7] clarifies why these tumors as reported in African-American women tend to be numerous and generally grow to varying large sizes often in the same uterus. In the pursuit of characterizing the genetic profile that directs leiomyoma size and morphogenesis, microarray technology utilizing DNA for gene expression measurements now occupies part of the forefront in research on leiomyoma tissue. This analysis attempts to identify the genes that are differentially expressed in leiomyomata as compared with normal myometrium.[16-19]

Wei et al.[20] used this type of gene chip analysis to examine for tumorigenic factors that contribute to large-size myomas. Hysterectomy tissue with myoma and 'normal' myometrial were selected to specifically identify the genes involved with leiomyomata varying from 1.5 to 19 cm (mean size 5.77 cm). The investigators did not characterize the ethnic group from whom the specimens were harvested.[20] However, they determined that the genes regulating nonsex steroid hormonal factors appear to have a greater role in contributing to larger leiomyomas.[20] This observation is not apart from the ovarian cycle steroid milieu. The response by leiomyomata to the changing hormonal environment includes but is not limited to the expression of specific growth factors, e.g., transforming growth factor-ß (TGF-ß), epidermal growth factor (EGF) and their receptors that direct cellular proliferation. There are other factors regulating cell death, e.g., Bcl-2 (antiapoptotic protein) that is upregulated by progesterone and tumor necrosis factor-α (TNFα) that is downregulated by progesterone. Consequently, correlating the hormonal status, i.e., phase of the ovarian cycle by histology or serum levels with specific peptides that influence the development of leiomyomata is relevant when linking repetitive chromosomal rearrangements, phenotypic characteristics and the genes identified by microarray technology that direct and/or promote the pathobiology of a leiomyoma.

Recently, Sandberg[21] provided an update on the cytogenetics and molecular genetics of leiomyomas. The most commonly identified chromosomal rearrangements in leiomyoma tissue include: translocations between chromosome 12 and 14-t (12; 14) (q14-q15; q23-q24), deletion (7) (q22q32) and rearrangement of chromosome 6-6p21. Less commonly observed are karyotypic abnormal rearrangements of 1p36, 3q, 10q22, 13q21-22, trisomy 12 and the X chromosome. Although rearrangements in either the short or long arm of the X chromosome have been found in leiomyomas, the preferred region appears to be Xp11~p22.

Sandberg[21] has a comprehensive list of the current cytogenetic abnormalities in leiomyomas. His review delineates specific cytogenetic subgroups and genes involved in leiomyoma formation as well as correlating genotype with myoma phenotype. His reported data is still silent on whether there is a preponderance of a specific genotype peculiar to African-American women. Furthermore, Sanberg characterized specific chromosomal genotypes associated with large myomas t (12; 14) and small myomas Del (7). The extracellular matrix (ECM), an area of leiomyomata that contributes to their size, is not discussed. This extracellular composition is the reason why these tumors are often called "fibroid."

The dense pseudocapsule surrounding the myoma

Figure 2. Normal uterus and pelvis

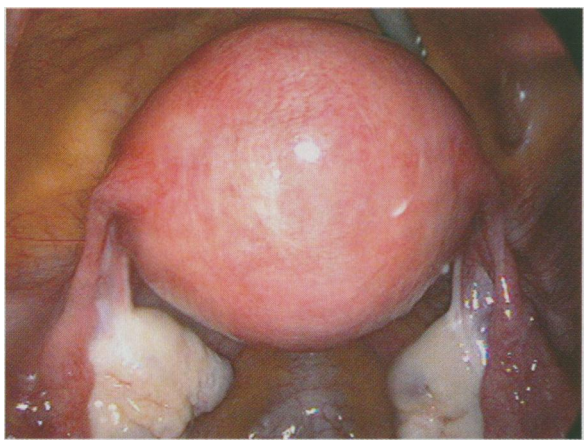
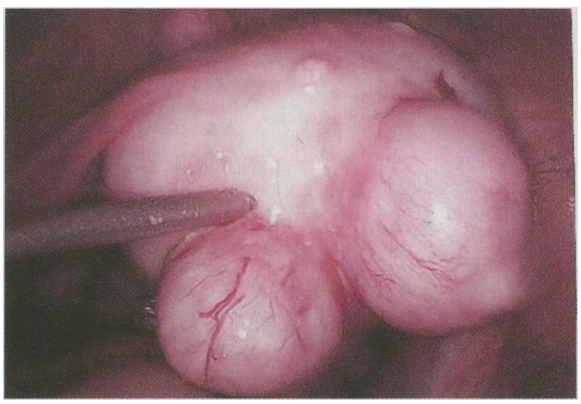


Figure 3. Interstitial or intramural myomas and subserosal myomas



nodule consists primarily of collagen, proteoglycan and fibronectin. Recently, Segars et al.[22] presented their findings at the 2nd NIH International Congress on the Advances in Uterine Leiomyoma research. Using microarray chips, this investigator and his colleagues compared the genes from "normal" myometrium and leiomyomas obtained by sampling uteri from five hysterectomies. Several key quantitative differences were noted between the genes arrayed from "normal" myometrial and leiomyoma tissue harvested. Among their findings, they indicated that while the extracellular protein, dermatopontin, a 22kd extracellular protein known to bind the collagen-binding protein decorin, is reduced, there is an increase in transforming growth factor-β (TGF-β) in the leiomyomata tissue. This is significant since dermatopontin has been documented to be reduced in hypertrophic and keloid scars. These are two disorders of tissue remodeling in skin prevalent in African-American women[23] and TGF-β is a key factor in the ECM. The investigators[23] presented an alternative hypothesis for the development of leiomyomas. They suggest that normal MSMCs undergo alteration in response to disordered extracellular signals arising from transformed myofibroblast.[24] Confirmation of these findings would likely support considerations for clinical trials of antifibrotic agent(s) potentially adding to the limited nonsurgical management of leiomyoma.

Sandberg's[21] update on cytogenetics analyses coupled with the focus on identifying the genes involved in myoma formation will undoubtedly provide us a better understanding of the knowledge required to decipher the pathobiology of leiomyomas. Obviously, research in key ethnic group(s) will accelerate the completion of this task.

The consistency of reports[25,26] on the high prevalence rates of leiomyoma—51% of premenopausal women, approximately 70% of Caucasian women and >80% of African-American women develop myoma by the time they reach menopause—raises questions. For example, apart from the cluster of familial predisposition to leiomyomata, "is the myoma the final common pathway for normal MSMCs' response to stimuli or are there other factors which have influence at different levels in affected ethnic groups?" This question follows from the immunohistochemical determinations by Lessey[27] and confirmation by the immunoblot analyses of Andersen.[28] They report that the level of ERs in normal MSMCs oscillates in the myometrium during the ovarian cycle. They determined that there is a gradual rise in myometrial ERs and progesterone receptors (PRs) levels during the mid-to-late follicular and during early luteal phase ER declines and dependent on the tissue studied (glandular epithelium, stroma or myometrium); PR declines as well except in myometrium and stroma. This segregation of function is linked to the effect of progesterone on both ER and PR and indicates that functional differences are mitigated in part by the tissue analyzed. Thus, the myometrium by the mid-luteal phase, during progesterone dominance, represses the transcriptional expression of the ER, while myometrial PRs content remains significant into the follicular phase.[27] It seems clear that the myometrium, like the endometrium, expresses cyclic changes at the molecular level that are regulated by estrogen and progesterone in preparation for pregnancy. The evidence will indicate that transformed myocyte, though similar to its normal progenitor cell, retains high levels of the PR concentration *throughout* the ovarian cycle and has elevated levels of ERs at the beginning of the follicular (proliferative) phase.

Given that leiomyomata are common and their growth linked to the reproductive years of women, it is ipso facto that they are rare prior to menarche and generally regress after menopause. These clinical observations highlight the impact of hormones on leiomyoma development. But since the pathogenesis of a leiomyoma appears to be similar to most neoplastic tissues, i.e., growth from dysregulated cell cycle progression, the phenomenon that the

Figure 4. Subserous myomas

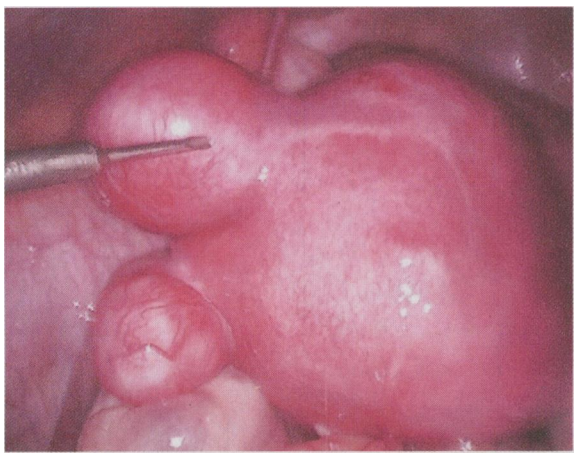
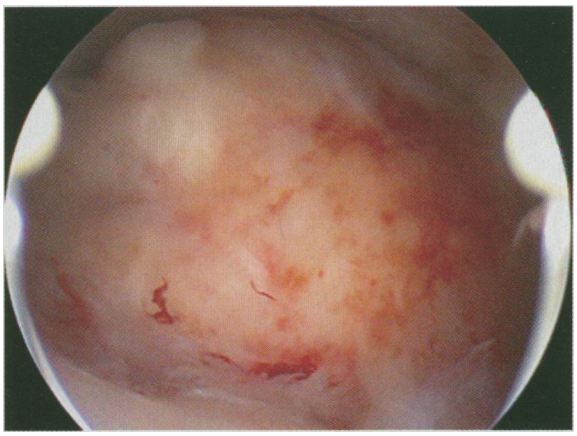


Figure 5. Submucous myoma, posterior wall



overwhelming majority of clinically detected leiomyomata remains benign raises other questions. What is the natural history of a leiomyoma? What factors promote leiomyoma development and preclude malignant transformation? This review will not examine the molecular dynamics involved with malignant transformation.

The prevalence of leiomyomata during the premenopausal years and the finding that the overwhelming majority of these uterine lesions are benign and asymptomatic have led to the practice of following these tumors clinically. Some have taken this practice into the realm of benign neglect, leading on occasion to unsettling surgical options. Nonetheless, the prevailing clinical dogma is to monitor the clinical impact and progression of documented leiomyomata. There are few studies[29-33] that have followed a cohort of women with leiomyomata to determine the natural history of growth and its correlation with symptoms; for example, bleeding, pelvic pressure symptoms. The ultrasound and magnetic resonance imaging (MRI) instruments are the two principal nonsurgical tools used to identify, characterize and monitor leiomyomas in symptomatic and asymptomatic patients. The Uterine Fibroid Growth Study[29] evaluated 120 women with leiomyomata a minimum of 2–5 cm in diameter as determined by ultrasonography. The average age of this cohort was 39.1 years old (range 24–54). Each participant was then monitored using the MRI over the course of one year. The ethnic distribution of the participants included 48% African Americans, 41% Caucasians, 4% Hispanics, 1% Asians, 1% native American; and 8% identified themselves as "other." One of the hypotheses tested in this study was that uterine leiomyoma are heterogeneous in terms of their growth. The investigators found intramural leiomyomata increased in volume at a slower rate than leiomyoma located in the subserosa or submucosa. They also indicated *that the rate of myoma growth was similar among women of different ethnicity.*

Further, the rate of growth was not different between women who elected surgical management for their leiomyomata secondary to the typical symptoms associated with these tumors. In addition, histologically, large tumors had significantly more extracellular connective tissue than smaller tumors. Another investigation[30] determined the incidence rate of uterine leiomyomata in a cohort of 64 asymptomatic premenopausal women. The average age of the participants was 44 years old (range 33–56), and they were followed for an average of 2.6 years. More than 90% of the women in this investigation were Caucasian, consistent with the population of Iowa. The prevalence and incidence rates of leiomyomata were 27% and 13% per 2.5 years, respectively. The tumors grew at average of 1.2 cm per 2.5 years. The growth patterns varied greatly among the patients. A more recent study[32] is the first report of the natural history of leiomyomata in two groups: familial and nonfamilial cases. The patients were followed for 4.33 years. They concluded that it is more common to encounter multiple leiomyomata in subjects whose family history was positive for leiomyoma. These studies[29,30,32] used modern radiological tools to evaluate symptomatic and asymptomatic premenopausal women with leiomyomata over the course of 1–4.33 years. Other reports indicate that the general pattern seen in large cohorts is slow growth during many years in premenopausal female followed by an apparent rapid volume enlargement during the perimenopausal years.[31,33]

In spite of this general growth pattern for leiomyomata, the African-American women are more likely to require surgical management and experience in-hospital complications largely attributable to the difference in their uterine size and number of leiomyomas at an earlier age than other ethnic groups.[8,26,34]

Given the fact that cytogenetic abnormalities are common in ≥40% of leiomyoma cells[9,10] and dysregulation of several growth factors in leiomyoma tissue is common,[35]

Figure 6. Right intraligamentary myoma

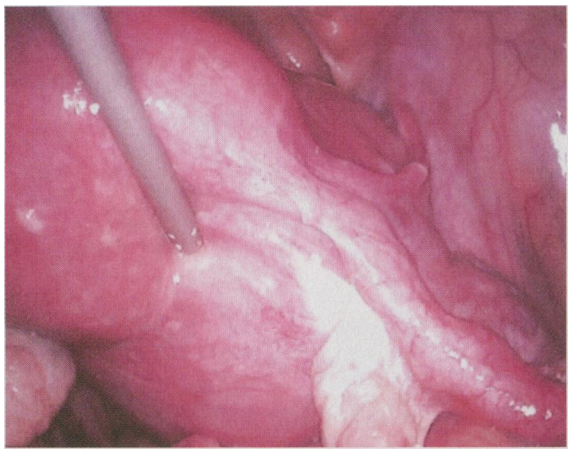
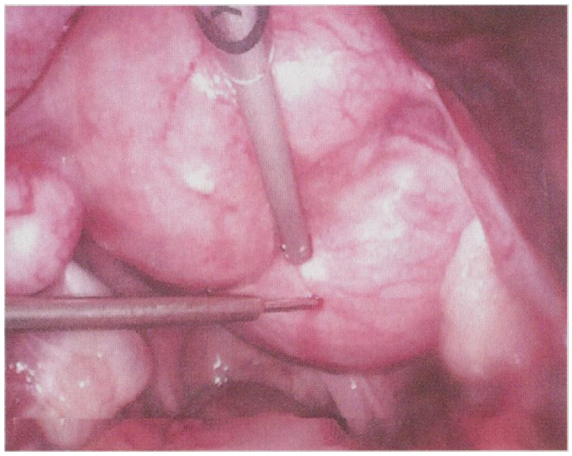


Figure 7. Right intraligamentary myoma



it is not surprising to note that there is a two-fold increased risk for developing leiomyomata among the first-degree siblings of women with leiomyomas as compared to unaffected women.[36] In support of a genetic predisposition, a mutation in the *fumarate hydratase* (FH) gene has been linked to women who have a hereditary form of uterine leiomyoma known as hereditary leiomyomatosis renal cell carcinoma (HLRCC).[37] This enzyme, FH, from the Krebs cycle, catalyzes the conversion of fumarate to malate. At present, the preliminary evidence suggests that identification of this mutation is of some predictive value among Caucasian women, but it has limited prognostic value, in African-American women.[36] Twin and family studies as well as several rare syndromes characterized by leiomyomas and other benign neoplasms are well documented in several reports that elaborate specific molecular and cytogenetic abnormalities associated with the development of leiomyomata.[14,21]

In summary, although there is a high prevalence of this condition, the true prevalence of leiomyomata in different ethnic groups is unknown for the same reason that the true incidence remains unknown; they are largely asymptomatic during the earliest stages of development. Secondly, there is no data to support any change from the current management strategy for patients with asymptomatic leiomyomata. Furthermore, there are no useful clinical data on the natural history of leiomyomata in different ethnic groups that would permit any definitive conclusions regarding altering current management based on ethnicity except for the African-American female who is at risk.

Although it is not a clinical dictum at this time, epidemiological data support that the standard should become early intervention in the African-American female during her reproductive years. The question is, are there unique epidemiological factors or pathobiologic event(s) in the uterus of African-American women that explain their peculiar predisposition for developing leiomyomata when compared with other ethnic groups?

EPIDEMIOLOGY

Leiomyomata appear to be less prevalent in European populations[38-40] as well as in Asian[41] and Hispanic[42] women. Among Nigerian women, a study reporting on a 10-year clinical review of management for uterine fibromyoma at the Ilorin Teaching Hospital (Ilorin, Nigeria) noted that 13.4% of new gynecological cases were admitted because of this diagnosis.[43] The prevalence of associated clinical conditions—pain, abnormal menstrual bleeding and infertility—was similar to what is seen in other parts of the world. Among American women, cases[42] comparing the prevalence of uterine myomas diagnosed by ultrasonography or hysterectomy indicated that both Hispanic and Asian women have similar risks to Caucasian women. In this country, symptomatic leiomyomata have a tremendous impact on the reproductive care of premenopausal women. Many studies are in agreement that African-American women are disproportionately burdened by the clinical sequelae associated with leiomyomata. These studies are consistent in finding an increased prevalence of "fibroid" in African-American women.[44-47]

At the 2nd NIH International Congress on the Advances in Uterine Leiomyoma Research in 2005, several reports reconfirmed the fact that African-American ethnicity is a risk factor in the development of leiomyomata independent of other variables. The exact incidence of leiomyomata uteri remains to be determined. The diagnosis depends on the method used to identify the condition.[44-58] Small myomas are clinically asymptomatic. Cramer and Patel[49] determined the incidence of myoma based on pathology specimen. They described leiomyomata in 77 of 100 consecutive hysterectomy specimens, when the uteri were sectioned at 2-mm intervals. Data from the Nurses Health Study II confirm that the incidence of leiomyomata among premenopausal women is high[52] [RR 3.25 (2.71–3.38)]. This risk persists even after controlling for other variables often related to race, including BMI, age at first birth, years since last birth and age when oral contraceptives were first used. Wise et al.[53] focused on risk factors specifically among Af-

Table 1. Key risk factors associated with leiomyomas†

Factor	Risk	Reference #
African Americans	Increased	44–47
Age (>30)	Increased	48,53, 56,57
Early menarche	Increased	8, 56
Nulliparity	Increased	8, 47, 48, 51
Multiparity	Decreased	51, 55
BMI ≥30	Increased/decreased	8, 41, 51, 56
Hormonal contraceptives	Decreased/null effect	8, 48, 51, 56
Tobacco	Decreased	48, 53, 56
Menopause/HRT	?	8, 56, 59
Diet	?	60, 61

BMI: Body mass index; HRT: Hormone therapy; † Adapted with permission from Flake, et al.[8]

rican-American women. Similar to the Nurses Health Study II,[52] Wise et al.[53] indicate that risk was inversely associated with age at menarche, parity, age of first birth and current use of injectable contraceptive. Baird et al.'s[47] subanalysis of myoma phenotype as multiple or single revealed that 73% of African-American women had multiple leiomyoma, whereas 45% of Caucasian women demonstrated this phenotype. This subanalysis supports previous data when comparing racial groups of women undergoing myomectomy or hysterectomy.[50,54,55] Myomectomy data[55] suggest that the myoma from African-American women are larger and more numerous than among a cohort of Caucasians. Kjerulff et al.[54,55] reported that African-American women have a greater risk of hysterectomy for symptomatic leiomyomas (65.4% vs. 28.5%) when compared with Caucasians.

Further, these investigators indicate that African-American women, compared with Caucasian women, undergoing a hysterectomy were more likely to have leiomyomata (89% vs. 59%), and were younger at the time of diagnosis (37.5 vs. 41.6) and at their age of hysterectomy (41.7 vs. 44.6). In addition, these investigators[55] provided data indicating that African-American women have significantly worse disease at the time of hysterectomy as measured by several endpoints—mean uterine weights (420.8 g .vs. 319.1 g), a greater likelihood of ≥7 tumors (57% vs. 36%) and an increased likelihood of anemia at the time of surgery (56% vs. 38%). Marshall et al.[44] and others[46,50,55,57] have concluded that African-American women tend to have larger size uterine leiomyomata. Recently, Wise et al.[48] in data derived from the Black Women Health Study reported a later peak incidence for self-reported cases of leiomyomas confirmed by sonography, hysterectomy or pelvic exam [45.6 (95% CI: 42.0–49.5)].

HORMONE AND DIET

Several investigators have examined the potential role of hormonal contraceptives,[53,56,58] hormone replacement therapy[56,59] and diet.[60,61] Oral contraceptives as well as the current use of progestin-only injectables are associated with a reduced risk for developing leiomyoma. Prolonged use of oral contraceptives may have a greater impact in reducing the risk, but this is not clear.[58] Current use of progestin injectables is associated with a 40% reduction in risk (95% CI: 0.4–0.9).[53]

Postmenopausal women with leiomyomata are potentially at an increased risk for surgical intervention. This observation has been linked to the dose and duration of progestin used. Palomba et al.[59] determined that after one year of hormone therapy with estradiol 2mg and medroxyprogesterone acetate (MPA) 5mg, there was a statistically significant change in mean leiomyoma volume as determined by sonogram when compared to the 2.5 mg of MPA with 2 mg of estradiol. The interaction(s) between diet and hormone metabolism has also been analyzed. In a case-control study, Chiaffarino et al.[61] concluded that Italian women who consumed more meat products (e.g., beef, ham) were at a greater risk for developing myomas when compared with those who ingested green vegetables. These investigators did not provide any estimates on dietary fat or caloric intake. Links exist between diet and hormonal metabolism. Woods et al.[60] confirm one aspect of this link by measuring the circulating levels of estrogens in African-American women compared to Caucasians. They concluded that African-American women have higher baseline circulating levels of estrone, estradiol, free estradiol and androstenedione compared with the Caucasians women in the study, while on a control diet (high fat, low fiber). Sex hormone-binding globulin levels were not different. When both groups were subjected to an experimental diet (low fat, high fiber) the circulating levels of estrogen was decreased. A similar observation was recently documented by Jung et al. in Korean women.[62] These investigators compared premenopausal females with leiomyomata with healthy matched controls. They were interested in the urinary concentrations of various estrogens, androgens and their metabolites. They reported that the patients with leiomyomata had increased levels of urinary concentrations of estrogens and androgens.

The reproductive years of women and African-American ethnicity are predisposing risk factors for developing uterine leiomyomata. Additional risk factors are listed in Table 1 (adapted with permission from Flake et al.[8]). However none of the factors listed explains the consistent ethnic differences that exist in the incidence, prevalence and size of myomas when African-American and Caucasian women are compared.[44,57] Since leiomyomata continue to impose a significant healthcare burden on women during their reproductive years, strategies for intervention will require accurate understanding of the natural history of the presenting phenotype and the pathobiologic pathway(s) that prescribes their growth and development.

The focus of current research is to understand the intracellular growth directives of leiomyoma cells by examining the genes and metabolic pathways associated with their development as compared to the "normal" adjacent myometrium. The expectation is that this effort will yield management tools that would safely permit early intervention and thereby arrest and/or reverse the clinical symptoms and conditions commonly seen from large and growing leiomyomas. The following will summarize and comment on key basic and translational research that characterizes the biology of leiomyoma development. Since the size of a leiomyoma, phase of the endometrium, areas of the leiomyoma accessed for study and ethnicity clearly influence the clinical presentation and conclusions regarding the biology of myomas, these aspects should guide our focus in deciphering mechanisms for leiomyoma development.

PATHOBIOLOGY

Estradiol and Progestin

Estradiol. The research data linking gonadal steroids to the development of leiomyomas span several decades and at times provide results that appear to be contradictory. Nonetheless, clinical and basic researchers have confirmed that ovarian steroids, specifically estradiol and progesterone, have a role in the development of a myoma. The clinical observation that ovarian steroids affect the growth of leiomyomas is evident by the following: they are rare during the prepubertal years; they grow,[63] diminish[64-66] or show minimal change during pregnancy[66,67] and generally regress in the postreproductive years.[68]

Investigators in the past decades have compared the circulating steroid levels[69-71] in subjects with myomas and controls, and measured and compared intramyoma steroid[72-75] and receptor concentrations for both estradiol and progesterone in "normal" myometrium and leiomyoma tissues from uterine specimens.[12,28,75-94] Generally, these data indicate there are consistent quantifiable differences between "normal" myometrial and leiomyoma tissue concentrations of gonadal steroids—estradiol, progesterone and their respective receptors. Investigators have also examined for cellular proliferative and apoptotic factors in leiomyoma as well as key metabolizing enzymes, e.g., aromatase 17β-hydroxysteroid dehydrogenase and estrone sulfatase. The operational mechanisms through which estradiol and progesterone cellular functions are expressed within a target cell have been determined. They briefly bind with specific nuclear and extranuclear receptors. The activated hormone receptor complex then binds to specific promoter regions of the target-cell DNA to effect gene transcription. The result within a leiomyocyte is a consequence of the net balance between cellular proliferation and programmed cell death (apoptosis).

When the circulating levels of ovarian steroids in subjects with leiomyoma are compared to normal women without documented leiomyoma, Spellacy[69] and others[70,71] have demonstrated that these levels are similar. These findings[69-71] coupled with consistent observations from operative and pathology reports indicate that leiomyomata from the same uterus have varying dimensions. This implicates local intrauterine factors in the development of myoma. This fact is highlighted by the paucity of studies correlating leiomyomata in subjects with chronic estrogen exposure, e.g., polycystic ovarian syndrome.

Once the ER, ER-α, was identified in leiomyoma cells,[76] researchers coupled the large body of clinical evidence correlating the apparent growth-promoting effect of estrogen on leiomyoma tissue to fuel the hypothesis that leiomyomas are estrogen dependent. The reports by Farber et al.[77] and Wilson et al.,[78] indicating that there are significantly higher concentrations of ERs in leiomyomas when compared with autologous myometrial tissue, have been convincingly confirmed by several independent investigators.[79-87] This difference in the quantity of steroid receptors between "normal" myometrial and leiomyoma tissue is a recurring finding that is not limited to the classic ER ER-α. The second subtype of the ER, designated ER-ß, appears to have mRNA levels preferentially localized in leiomyoma tissue when compared with matched myometrium.[89,90] There are two independent chromosomal locations for both isoforms of the ERs in humans.[88,95] Recently, Sakaguchi et al.[96] have reported that both isoforms of ER-mRNA change in a similar manner in the myometrium during the menstrual cycle; however, ER-α mRNA levels are greater than ER-β mRNA.

Although several studies indicate the presence of ER-ß in leiomyoma tissue,[96-99] ≥1 report[92] indicates that only myometrial and leiomyoma microvascular endothelial cells express ER-ß, whereas cultured myometrial and leiomyoma smooth-muscle cells generally express ER-α but not ER-ß. Jakimiuk et al.[97] demonstrated in premenopausal women from Poland that the expression of ER-α and ER-ß did not differ between leiomyomas and matched myometrium, yet ER-α expression was significantly higher than ER-ß in both tissues. Similar findings were reported by Lessl et al.;[100] however, they did not measure both isoforms of the estrogen or PRs. Amant et al.[83] examined leiomyoma and adjacent myometrial tissue from blacks and whites in South Africa to determine if ethnic variations in the estrogen and PR levels could explain the greater prevalence of the condition in the black females in South Africa. They concluded that the differences in leiomyoma expression between the two ethnic groups could not be attributed to the ER, PR-A/PR-B or myometrial ER-α mRNA levels. However, these investigators noted the lack of a systematic registry for the biopsy site. Further, they did not provide any information regarding uterine weight or leiomyoma size. Dating of the endometrium was provided to distinguish tissue from the proliferative and secretory phases. Consistent with these findings and Cargett et al.[92] is a recent retrospective immunohistochemistry analysis designed to determine the presence of ER-α and ER-ß in the cells of connective tissue and the subcellular localization of these receptors in leiomyoma and myometrial tissue from the same uterus.[101]

Valladares et al.[101] reported that the immunoreactivity of the ER-α is almost exclusively in the smooth-muscle cells of leiomyoma tissue in either the nucleus or cytosol. The ER-α analysis in normal myometrium did not show significant differences in this receptor distribution when compared with the smooth-muscle cells of leiomyoma tissue. The immunoreactivity for the ER-ß indicates a lower and variable presence in smooth-muscle tissue of myometrium or leiomyoma tissue. However,

ER-ß was always present in the nuclei of cells from connective and endothelial leiomyoma tissues without evidence for ER-α immunoreactivity. Relatively contrary to these findings, Wei et al.[102] reported at the 2nd NIH International Congress on the Advances in Uterine Leiomyoma research an abstract regarding ethnic differences in tumorigenic factors in uterine leiomyomas. Their objectives were to identify selective genes by tissue microarray analyses and specific immunohistochemistry determinants involved in the development of leiomyomas and compare results to matched myometrial tissue. Four ethnic groups were examined: African Americans, Asians, Hispanics and Caucasians. They indicated that PR-A was upregulated in leiomyoma tissue of African- American women compared with the other ethnic groups. Furthermore, they reported that the ER-α was elevated both in the "normal" myometrial and leiomyoma tissues of African-American women when compared with the other groups. These investigators published their completed findings correlating leiomyoma size, phase of cycle and matched myometrium.[103] Despite the apparent lack of scientific consensus on the distribution of ER-α and ER-ß in leiomyoma and myometrial tissue, it should be noted that the mapping of ER-β to 14q22-24 by Enmark et al.[95] is close to one of the more common chromosomal rearrangements in leiomyomas, t (12; 14).

Progesterone. It has been determined that the mitogenic effect of estrogen on leiomyoma tissue is mediated through the up regulation of growth factors[35] and in part from the induction of the PRs.[3,4] Tiltman, examining pathology specimens of leiomyoma tissue for mitotic numbers, determined that the highest mitotic counts/high-power field was associated with a progestin-only preparation (depo-medroxyprogesterone acetate) when compared with controls and the combination oral contraceptive agent.[104] The addition of estradiol or progesterone to cell cultures of myoma results in an increased mitotic rate of leiomyoma tissue.[105] The observation in cultures of *normal* myometrial cells indicates that only estradiol elicits a proliferative response. The conclusions of Lessey et al.[27] and Andersen et al.[28] have been corroborated. That is, the sequential presentation of ovarian hormonal secretion is associated with an increase in both the estrogen and PRs in normal myometrium[84,93,105] and leiomyoma tissue.[105-108]

Both isoforms of the PR—PR-A and PR-B—are expressed from two promoters on a single gene.[109] Whereas PR-A appears to function as a transcriptional inhibitor, PR-B functions as a transcriptional activator of progesterone responsive genes.[109,110] In addition, there is complex "cross-talk" between the intracellular signaling pathways for the ERs and PRs.[111-113] In the majority of the literature based on the use of mRNA levels by radioligand binding assays, in situ hybridization, western blotting techniques and immunostaining quantitative analyses with myometrial and leiomyoma tissues, the receptors' concentrations are different in leiomyoma tissue when compared with adjacent myometrial tissue from the same specimen. Andersen and Barbieri[12] characterized the typical pattern of the acquisition of both receptors (ER/PR) in normal myometrial tissue during the follicular phase of the ovarian cycle, and this process is maintained into the early luteal phase. Subsequently, during peak levels of progesterone and into the late luteal phase, lower levels of each receptor are present. Thus, at the beginning of the menstrual cycle, both ER and PR are at reduced levels in normal myometrium. This oscillating pattern in normal myometrial tissue is absent in leiomyomas. Rein et al.,[3] indicated that leiomyoma tissue from the follicular phase binds more progesterone than similar tissue from women during the luteal phase. When we consider Tiltman's[104] study, that increased mitotic numbers are highest in leiomyoma tissue when exposed to progestins, the work of Rein is better understood. It would seem that the influence of circulating ovarian hormones is of relative importance once a myocyte alters its response to the dominant ovarian hormones and acquires in situ estrogen[72-75] synthesis capacity. Further review of the role of progesterone in leiomyoma tissue could lead to the conclusion that progesterone, and not estrogen, is more important to the development of leiomyomas. One of the several genes that are upregulated by estrogen is the PR. Rein et al.[3,4] presented clinical, pathological and molecular biochemical evidence suggesting that progesterone, progestins and the PR promote leiomyoma cellular proliferation. Several investigators have demonstrated an increased concentration of both isoforms of PR in leiomyoma tissue when compared with adjacent myometrium from the same uterus.[87,94,106] The observed utility of mifepristone in reducing leiomyoma volume by Murphy et al.[114] confirms the effect of progestin on the cellular function of established leiomyoma cells in vivo. Wei et al.[115] have convincingly established a vital role for progesterone in the development of leiomyomata. They showed by in vitro analysis that a selective PR modulator (asoprisnil) can inhibit leiomyomata cellular proliferation and induce apoptosis without any comparable effects on cultured normal myometrial cells.

Leiomyoma tissue has significantly higher levels of epidermal growth factor (EGF) mRNA than myometrium during the secretory phase of the menstrual cycle. EGF plays a crucial role in regulating leiomyoma growth. It has been postulated that both estradiol and progesterone coordinate their effects on EGF by upregulating EGF receptors and EGF-like proteins, respectively.[116] The anti-apoptotic protein, Bcl-2, is overexpressed in leiomyoma tissue relative to adjacent myometrium. The addition of progesterone to cultured leiomyoma cells increases Bcl-2, whereas the addition of estradiol results in lower levels of Bcl-2.[116] Since Bcl-2 promotes cell survival by preventing apoptotic cell death, proges-

terone in addition to its effect on EGF mRNAs regulates leiomyoma growth through the expression of Bcl-2. The downstream effects of progesterone intracellular actions extend to the extracellular matrix through transforming growth factor-ß (TGF-ß) and in addition to upregulating Bcl-2; it suppresses tumor necrosis factor-α (TNF-α).

TNF-α has the ability to induce apoptosis in various cell types. Kurachi et al.[117] were first to demonstrate that the immunoreactivity of TNF-α in leiomyoma is higher than in myometrial cells. Kurachi et al. demonstrated that there is no significant difference in TNF-α immunostaining between the proliferative or secretory phase of myometrial cells in vitro. The addition of estradiol does not affect TNF-α expression. However, the addition of estradiol and progesterone does augment the intensity of cells stained for TNF-α.

The recent review by Sozen and Arici on the various cytokines and growth factors that are responsible for mediating the effect of the ovarian steroids in leiomyoma tissue concluded the following: "A review of the literature reveals that TNF-ß is the only growth factor shown to be overexpressed in leiomyomata versus myometrium, hormonally regulated both in vivo and in vitro, and both mitogenic and fibrogenic in these tissues."[118] This conclusion is supported by the findings from the microarray analysis by Catherino et al.[23] These investigators expected that estrogen upregulated genes would be predominantly characterized. However, it is the genes encoding proteins from the ECM that were overexpressed. The collagen-binding protein dermatopontin was found to be reduced in leiomyoma tissue, whereas TGF-ß3 mRNA levels were increased. Among the various cytokines and growth factors associated with the cellular proliferative process of leiomyoma growth, only TGF-ß has been documented to have a significant role in the accumulation of ECM.[119] Arici and Sozen have determined that at present, the only growth factor that is overexpressed in the leiomyoma samples during the secretory phase is TGF-ß3.[120] However, lest we conclude that the progesterone effect on leiomyoma tissue is only mitogenic; in vitro studies indicate that it may also exert an inhibitory effect of cellular proliferation through the downregulation of insulin growth factor-I (IGF-I) mRNA levels. While this is so based on protein analyses for mRNA levels, neither estradiol nor progesterone affects IGF-I receptor mRNA expression. In addition to these steroidal effects on IGF-I, as reported by Maruo et al.,[116] they indicate the IGF-I can serve as a surrogate for estrogen not only in mediating mitogenesis in leiomyoma tissue but also in inducing the PR. The net effect of the dual actions of progesterone on leiomyoma tissue seems to be mitogenic. However, the precise intracellular mechanism that directs intracellular signaling pathways for specific cellular action, the cross-talk between steroids and their receptors, remains to be clarified.

Recently, alternate approaches have been explored to ascertain the basis for the increased prevalence of leiomyoma in African-American women. Investigators studying black South-African women examined for polymorphism (chromosome allele variation) in the gene, CYP17, that regulates 17α hydroxylase and 17, 20 desmolase.

These are metabolic enzymes in the $\Delta^{5,4}$ steroidogenic pathway.[121] In this study, the investigators extrapolated from their data of healthy nulliparous premenopausal females ages 18–33 from different ethnicities. They concluded that there is a "stronger estrogenic" effect on the uteri of women who are homozygous for cytochrome P450c17α (CYP17) genotype–A2 allele as compared with the A1 allele, heterozygotes.[122] Relevant hormonal measuring is lacking in this study. The difference in A1 allele and A2 allele results from a single nucleotide change in (1) base pair (1-bp). When nucleotide thymidine is present, the designation is A1 and if nucleotide cytosine is present, the designation is A2. Fiegelson et al.'s[122] data provided direct evidence of genetic control of serum hormone levels. Individuals with the A2/A2 genotype were documented to have statistically significant increased circulating levels for estradiol and progesterone on days 11 and 22 of the cycle when compared to individuals with A1/A1 genotype. Amant et al.[121] examined black and white South-African women who had undergone hysterectomy for symptoms associated with leiomyomas (affected) or hysterectomy for nonleiomyoma pelvic pathology (controls). The specific pathology of the control group in not described. The study attempted to link polymorphism in CYP17 genotype to subjects with and without leiomyomas. They found that none of the subjects in the black South-African controls were homozygous for A2 allele, whereas 16.9% of the affected black women and 9.5% of the affected Caucasians women were homozygous for the A2 allele. They did find that 3 out of 35 white controls were homozygous for the A2 allele. Although steroid levels were not measured, their statistical analyses suggested that CYP17 A2A2 genotype is significantly linked to the development of leiomyomas in South-African black women. The analysis is not significant when the same genotype in the white South-African women with leiomyomas is tested. They reasoned that the leiomyomas in their black South-African women developed because of the homozygosity for CYP17 and the presumption that there were elevated circulating levels of the dominant ovarian steroids in their population. However, this assumption regarding the steroid levels is based on the earlier findings in African-American women as reported by Woods et al.[60] and Fiegelson et al.[122] Other investigators[123,124] have explored whether single-nucleotide polymorphisms (SNPs) could provide further insights into the etiology of this benign neoplasm.

Denschlag et al.[123] hypothesized that ≥1 proestrogenic SNPs is associated with leiomyomas. They chose

three SNPs that are known for their modulation of estrogen action. Their population consisted of 130 women with leiomyomas and 139 controls without leiomyomas. The women were Caucasian, primarily of German ethnicity, with rare minority immigrants. The SNPs investigated for their associations with uterine leiomyoma were: ERα, catechol-O-methyltransferase (COMT) and CYP 17α genes.

Variations of COMT based on a single nucleotide change can either retard estradiol metabolism, causing higher intracellular levels, or significantly reduce the levels. Thus, an investigation of the effect of these SNPs and leiomyoma is useful. Similar to Amant et al.,[121] who demonstrated no statistically significant correlation in white South-African women with leiomyoma and the presence of the A2 allele for CYP17, Denschlag et al.[123] confirmed the same in the 130 women with pathologically confirmed leiomyomas. They did not demonstrate that any of the SNPs investigated alone or in combination were significantly associated with leiomyomas in the Caucasian population studied when compared with the control group. This same finding regarding ERα was reported by Massart et al.[124] for Italian women. Their study also concluded the same for ERß and the PR. This is contrary to the work of other investigators[125-127] who showed a significant association between ERα gene polymorphism as well as COMT and susceptibility to leiomyoma development. Hsieh et al.[125] indicate that specific dinucleotide repeat polymorphisms in Taiwanese women confer increased predisposition for developing leiomyomas.

Al-Hendy et al.[126,127] examined COMT and ERα for polymorphism in women from the following ethnic groups: African Americans, Caucasians and Hispanics. Women with the high-activity genotype for COMT convert 2-hydroxy estrogen ($2OHE_2$) into a methylated highly estrogenic compound 2-methoxy estrogen. These investigators hypothesized that women with leiomyomas are more likely to have the homozygous allele configuration for COMT. Their investigation revealed that women with the high-activity genotype for COMT are 2.5 times more likely [odds ratio (OR)] to develop leiomyomas than women with other genotypes. This finding was not tested in women without leiomyomas prospectively. Nonetheless, their analysis of the myometrial cells from different ethnic groups described the normal myometrial cells from African-American women exhibited a greater prevalence of the COMT genotype that is associated with higher estrogenic responsive elements. When this finding is coupled with their study on polymorphism for the ERα[127] in black Americans, it is evident that submicroscopic genetic anomalies may be operational at different levels in the African-American females predisposed to forming leiomyomas.

These studies[123-127] correlate DNA sequencing in peripheral blood samples with myometrial and leiomyoma tissue DNA. The results from these studies could lead to the affirmation that the estrogen hypothesis provides the most plausible biological explanation in support of African-American women's greater potential for developing leiomyomas. However, when the studies are carefully examined for methodology, there are findings of concern to note. For example, in Amant et al.,[121] the sample size of the population studied is too small to give us confidence in their statistical estimates. Although Denschlag[123] and Massart[124] reached similar conclusions, the controls in Massart's work were postmenopausal women with an average age of 63.6, while the study patients were premenopausal female with an average age of 48.6. They stated that the receptor analyses for polymorphisms were performed on 15–21 leiomyomas from each of five patients who underwent hysterectomy from a total of 413 Italian women. The phase of the endometrium was not mentioned, and the size of the myoma was not noted. In addition, in their analyses of the receptors of study, there were no validation studies reported, i.e., protein isolation and analysis and/or immunohistochemistry analyses with specific antibodies to the receptors. Finally, the site of biopsy from the myoma nodule was not noted. These observations limit any conclusion regarding the genesis for growth and development of leiomyomas in African-American women.

Careful examination of the intratissue levels of specific enzymes involved in leiomyoma proliferation has been explored in order to decipher the process of in situ estrogen production. These studies have focused on three enzymes—aromatase,[128-132] 17β-hydroxysteroid dehydrogenase[133] (17ß-HSD) and estrone sulfatase.[134] Except for the aromatase enzyme,[132,135] these enzymatic analyses reached conclusions pertinent to Asian women[128-130] and northern-European Caucasian women.[134] These studies examined for the tissue concentration of estrogen and other hormones in leiomyomas and the nonadjacent myometrial tissue (>2 cm away from the leiomyoma capsule). They have been consistent in finding that leiomyoma cells express two critical enzymes for the in situ production of estrogen—aromatase[128-132] and type 1 17 ß-hydroxysteroid dehydrogenase (17ß-HSD)[133]—whereas the nonadjacent myometrial tissue do not.

This selective growth advantage by leiomyoma cells[135] did not yield positive correlations among the aromatase mRNA levels and leiomyoma size, uterine weight or patient age. There was a positive correlation between the older age (>45) and aromatase mRNA levels. This finding may, in part, explain the observed gradual loss of myoma volume seen over time in the postreproductive years. No correlation analysis between the mRNA levels of type 1 17ß-HSD and leiomyoma or uterine weight was presented in the work of Kasai et al.[133]

CONCLUSION

There is unanimous consensus in the literature that premenopausal African-American women are disproportion-

ately burdened by the clinical conditions resulting from developing leiomyomas. There are no apparent epidemiological factors that explain this observation. The examination of uteri in this ethnic group for pathobiologic difference(s) from other groups through molecular profiling and other biochemical and cytogenetics analyses are still without definitive answers. Studies attempting to analyze for differences must consider multiple variables such as what part of the myoma node is selected to perform specific studies. The relevant observation by Bourlev et al.[136] is that leiomyoma growth is in the peripheral parts during the secretory phase. This finding was later confirmed through the immunohistochemical analyses performed by Pavlovich et al.[137] They demonstrated that the secretory phase is associated with the maximum proliferative activity in myomas and that the PRs at the peripheral and central parts of the myoma node are high in both the proliferative and secretory phase. Wei et al.[138] expanded on this work by determining the distribution of gene and gene products over selected zones from the periphery to the center of small (≤2 cm) and large myomas (≥10 cm) covering three separate axes of each myoma. The findings are consistent in that the peripheral part of the myoma is the most biologically active zone. One interesting determination is that the mean levels of ER and PR in large myomas are significantly lower than in small myoma nodes. This is consistent with their previous observations[139] and with the majority of microarray analyses indicating reduced expression for these receptors and possibly indicating that large myomas are likely not as dependent on the activity from high levels of ER and/or PR. However, the authors did not discuss differences among small myomas.

Many of the analyses prior to the current decade fail to indicate the part of the myoma extracted for tissue analysis. This observation applies to several of the receptor and mRNA studies conducted to date. The current emphasis of microarray analysis coupled with focused biochemical and molecular determinations should begin to illuminate the pathobiologic mechanism(s) that lead to the development of leiomyomas. The strategy involved in our current management relies heavily on surgical intervention. It is anticipated that armed with the knowledge from the human genome project and implementing early intervention for women at risk, the result should be a favorable impact on the quality of life wherein leiomyoma associated symptoms are reduced or eliminated.

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