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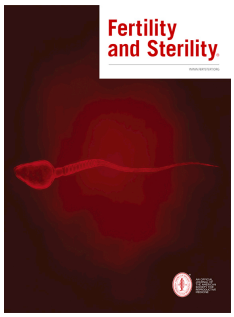

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Pathogenesis of uterine fibroids: current understanding and future directions

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Abstract

Fibroids are benign uterine tumors characterized by proliferation of uterine smooth muscle cells, embedded in an abundant extracellular matrix. Their prevalence is estimated to be more than 50% in women over the age of 45 years.

Fibroids represent a considerable health burden. It is time to acquire a deeper mechanistic understanding of uterine fibroid-related etiology and pathogenesis, which may help pinpoint new strategies and an individualized approach. There is a need to gather prospective data and conduct studies to compare alternative approaches and assess long-term outcomes with respect to quality of life, recurrence of symptoms (bleeding, bulk symptoms), fertility, and even complications The goal of this review is to evaluate the widely accepted pathogenesis and identify risks factors and future directions for clinical and basic research into fibroids.

Key words: uterine fibroids, pathogenesis, risks factors

Fibroids are benign uterine tumors characterized by disordered monoclonal proliferation of uterine smooth muscle cells, embedded in an abundant extracellular matrix (ECM) (1) containing collagen, fibronectin and proteoglycans. The prevalence of fibroids depends upon ethnicity (1, 2) and is estimated to be more than 50% in women over the age of 45 years (2-5). Some fibroids are asymptomatic, but others result in symptoms like heavy menstrual bleeding, pelvic pain, bulk symptoms and infertility (3,6,7). These manifestations impact the quality of life of affected women and certainly warrant therapy (2, 4, 6, 7).

Fibroids represent a considerable health burden. Current management strategies are mainly surgical and expensive (2). Of 600,000 hysterectomies carried out every year in the United States, one-third are for fibroids (8). As stressed by Dolmans et al (9), it is time to acquire a deeper mechanistic understanding of uterine fibroid-related etiology and pathogenesis, which may help pinpoint new strategies and an individualized approach. The goal of this review is to evaluate the widely accepted pathogenesis and identify risks factors and future directions for clinical and basic research into fibroids.

1- Pathogenesis

Uterine fibroids, or leiomyomas, are benign tumors in smooth muscle and connective tissue of the myometrium. They mainly contain myocytes and are characterized by excessive deposition of CM substances, primarily collagen (10).

The fact that ovarian steroid hormones play a major role in the pathogenesis of uterine fibroids is supported by epidemiological, clinical and experimental evidence. Estradiol and progesterone prompt mature leiomyoma cells to release mitogenic stimuli to adjacent immature cells, thereby providing these myomas with undifferentiated cells that are likely to support tumor growth. Progesterone action is required for complete development and

proliferation of leiomyoma cells. Sex steroids are critical to fibroid development and maintenance, but a number of autocrine and paracrine messengers are also involved in this process. Furthermore, estradiol and progesterone work together to maintain viability for tumor development, since estrogens increase the availability of progesterone receptors inside cells (Reis et al, 2016).

Uterine fibroids are considered fibrotic disorders because they contain 50% more ECM proteins than their corresponding myometrium. Excessive ECM accumulation and aberrant remodeling are central features of uterine fibroids. They are characterized by elevated levels of collagens, fibronectin, laminins and proteoglycans (11). They can also induce mechanotransduction by activation of the integrin-Rho/p38 MAPK/ERK pathway (12), resulting in altered bidirectional signaling between leiomyoma cells and the ECM. Growth factors (transforming growth factor beta [TGF-β], activin-A and platelet growth factor), cytokines (tumor necrosis factor alpha), steroid hormones (estrogen and progesterone) and microRNAs (miR-29 family, miR-200c and miR-93/106b) all influence ECM accumulation (Islam et al, 2018).

TGF-β 1 and 3 and activin-A are the main players in excessive ECM accumulation (fibrosis) and myofibroblasts in leiomyomas (13). Interestingly, the ECM may serve as a reservoir of profibrotic growth factors and enhance their activity by increasing their stability and extending their duration of signaling (14). Sphingosine-1-phosphate signaling, which is involved in multiple-organ fibrosis, is dysregulated in uterine fibroids and involved in growth factor-induced fibrosis 15).

Some evidence supports the hypothesis that uterine fibroids originate from myometrial stem cells (MMSCs), but the specific cell of origin has not yet been identified. One hypothesis from the team of Al-Hendy (17) points to genetic transformation of a single MMSC into a tumor-

initiating cell due to various factors, including genomic instability, inflammatory microenvironment, and developmental and environmental insults (17,18).

2- Risks factors

Risk factors for uterine fibroids are illustrated in Figure 1.

1) Age

By magnetic resonance imaging, the average growth rate was calculated to be 9% over the course of 6 months in a series of 72 women with uterine fibroids (n=262 fibroids), but growth rates differed between races when age was taken into account (19). Furthermore, delaying the first pregnancy until the third decade of life is known to place women at higher risk of myomas (20,21).

2) Race

Race constitutes an important risk factor for leiomyoma development (22-25). The incidence of uterine fibroids was found to be as high as 60% by age 35 among African-American women, increasing to >80% by age 50, while Caucasian women showed rates of 40% by age 35, climbing to 70% by age 50 (26). African-American women are clearly at greater risk of being affected by uterine fibroids, particularly at an earlier age (27,28). Moreover, recurrence rates after surgery (myomectomy) may be close to 60% after an interval of 4–5 years (2,29,30) in women of African origin.

3) Early menarche

Menarche at an early age increases the risk of developing fibroids and is also considered a risk factor for other hormonally mediated diseases, such as endometrial and breast cancers (24,31,32). It has been demonstrated that steroids, particularly progesterone and

progestogens, play a crucial role in the development of leiomyomas (2,33,34,35). By binding to cytosol and nuclear progesterone receptor, progestogens favor proliferation and inhibit apoptosis in uterine fibroids through a number of pathways, including the Akt pathway (35). Use of oral contraceptives can reduce the risk of developing fibroids (32).

4) Parity

Pregnancy has been found to have a protective effect against development of uterine fibroids, but the mechanism remains unclear. Indeed, nulliparous women are more often affected by uterine fibroids than multiparous women (32), with each subsequent child lowering the risk. It has been suggested that during postpartum uterine remodeling, small lesions may be subject to selective apoptosis. Fibroid tissue may also be highly susceptible to ischemia during both uterine remodeling and childhood (26,36). High progesterone exposure during pregnancy and its rapid withdrawal after delivery may also influence the uterine remodeling.

5) Caffeine and alcohol

An association was reported between alcohol and caffeine consumption and an elevated risk of developing uterine fibroids in a study into the health of women of African origin (23,28).

6) Obesity, hypertension, smoking

General health status may also be predictive of leiomyoma growth, with factors such as obesity and high blood pressure playing a part. Obesity is a significant risk for uterine fibroid development. It is attributed to the metabolic functions of adipose tissue, which releases growth factors and cytokines that may be involved in different pathological processes. Moreover, aromatase, present in adipose tissue, is responsible for conversion of adrenal androgens into estrogens (27). The greater risk of developing uterine fibroids in African-American populations in the United States is related to high rates of obesity and excessive body mass index, which are more prevalent among African-Americans than other racial

groups. Concerning arterial hypertension (37,38), there is an association between increased diastolic blood pressure and a higher risk of uterine fibroids. In fact, women suffering from high blood pressure are five times more likely to develop myomas. A diet rich in red meat appears to increase the risk, while smoking decreases the risk for unknown reasons (21,24,32,33).

7) Endocrine disruptors

Endocrine disruptors (EDs) are known to be linked to development of estrogen-dependent diseases like endometriosis (39). Recent reviews (16,21), have suggested their involvement in the pathogenesis of uterine fibroids (see below).

8) Genetic factors

Some specific genetic alterations are linked to fibroid growth (40-44). Mehine et al (40) performed whole genome sequencing and gene expression profiling of 38 uterine leiomyomas and their corresponding myometrium. The common occurrence of chromothripsis in uterine fibroids suggests that it also plays a role in their genesis and progression (40,42). So-called derived genetic alterations responsible for the formation of fibroids were identified thanks to genomic technologies. The most prevalent mutations were found to be in the Xq 13 gene encoding the RNA polymerase II mediatic subunit MED-12. High-mobility group AT-hook 2 (HMGA2) and collagen IVa5 (COL4A5) were the other two most frequently encountered somatically derived mutations (40). Mutations in fumarate hydratase on chromosome 1 were also found in leiomyomas (45).

3- New approaches to understanding the pathophysiology

1) MED-12 mutations

According to a recent meta-analysis (46), MED-12 mutations occur more commonly in women of African origin than in white populations. However, the molecular basis by which MED-12 mutations cause uterine fibroids and the high prevalence of MED-12 exon 2 mutations are not yet understood. Nonetheless, it is clear that these mutations interact with hormonal angiogenic and growth regulatory factors to favor fibroid progression.

Several studies (43,44, 47-54) have indeed revealed that MED-12 mutation-positive uterine fibroids are associated with smaller tumor size, conventional tumor histology, increased tumor number and subserous locations (55). In normal culture conditions (monolayer in vitro culture), primary cells from MED-12 mutation-negative uterine fibroids were able to survive for many passages, while those derived from MED-12 mutation-positive tumors were rapidly lost within the first few passages. This may explain why MED-12 mutation positive and -negative tumors might exhibit unique growth features (16,55).

According to Yang et al (16), MED-12 exon 2 mutations could possibly give a selective advantage to myometrial stem/progenitor cells by altering their growth and/or trajectory of differentiation, leading to formation of uterine fibroid stem cells. The pathogenesis of uterine fibroids points to genetic transformation of a single MMSC into a tumor-initiating cell that sustains clonal tumor growth through endocrine, autocrine and paracrine factors, and hormone receptor signaling (56). However, multiple genetic subtypes may be present in a uterus where fibroids arise independently and signaling pathways show substantial overlap, explaining why genotype-based therapies have not been developed (42). Moreover, multiple concurrent gene rearrangements and genomic instability also play a role in the pathogenesis of fibroids (40-42).

2) Endocrine disruptors

In the view of Yang et al (16), the adverse effects of environmental (exposure to EDs) and hence developmental insults may target MMSCs and alter their characteristics, which govern reprogramming of the epigenome and initiation of hormone-dependent uterine fibroid pathogenesis.

EDCs are present in plasticizers, dioxins, polychlorinated biphenyls, organochlorines, phthalates and diethylstilbestrol. Direct association of EDs with the estrogen receptor and epigenetic reprogramming of the developing uterus is one conceivable hypothesis, according to Pavone et al (21). Numerous EDs (57,58) can bind to nuclear receptors and consequently alter hormone functions by mimicking naturally occurring hormones in the body, thereby blocking binding of endogenous hormones. In a recent review, Jang et al (16) reported some molecular mechanisms underlying developmental ED exposure-induced risks of uterine fibroids. They concluded that this exposure epigenetically targeted MMSCs, leaving a hormonal imprint on key signaling pathways, which results in an increased risk of uterine fibroids in hormone-dependent women.

It is not ruled out that intrauterine and early-life environmental exposure to EDs may act as an 'early hit' by hijacking epigenomic plasticity to induce MMSC reprogramming. Reprogrammed cells could also be transformed into tumor-initiating stem cells after later-life adverse exposure in what Yang (16) calls the 'late HIT'.

3) Epigenomic and enhancer dysregulation

Mlodawska et al (59) recently reported the latest findings on epigenomic changes found in uterine fibroids: aberrant DNA methylation and histone tail modification. Such modifications in the promoter or enhancer regions can dysregulate gene expression, which helps to form the uterine fibroid phenotype. Application of an altered enhancer landscape to identify

consequences on gene regulation requires further investigation and should be considered a new approach to elucidate the development of uterine fibroids (59). According to Mlodawska et al (59), interplay of cell signaling pathways and histone modifications explains the altered 3D chromatin structure, which contributes to development of uterine fibroid-specific gene dysregulation, resulting in its pathogenesis. Dysregulated genes participate in tumor suppression, apoptosis, angiogenesis, ECM formation and stem cell differentiation. These recent findings may shed light on novel approaches on prevention and identification of epigenomic targets for long-term management of uterine fibroids. In their review, Mlodawska et al (59) acknowledge that while many molecular mechanisms and epigenomic features have been explored, reasons for the racial disparity remain unclear.

4) MicroRNAs

Among the numerous microRNAs (miRNAs) identified in uterine fibroids whose expression profiles differ from autologous myometrium, miR-21 and miR-29 play key roles (24). MiR-21 is overexpressed in leiomyomas by blocking the inhibitory effects of the Smad 7 protein. This stimulates the TGF-β signaling pathway, enhances expression of TGF-β in uterine fibroids and boosts collagen and cell proliferation (60). Knockdown of miR-21 in leiomyoma cells leads to increased apoptosis (61). MiR-29 is downregulated in fibroids and is a negative regulator of ECM genes, including collagens I, II and III (62).

5) Vitamin D

Vitamin D binds to its specific vitamin D receptor to mediate its functions through steroid transcriptional mechanisms (63) The action of vitamin D in uterine fibroids was recently reviewed by the team of Al-Hendy (16,64). Vitamin D inhibits TGF-β and is associated with its reduced expression and induced ECM proteins, including collagen type 1 and fibronectin in uterine fibroid cells, which are otherwise overexpressed (64). It also inhibits proliferation of

fibroid cells by downregulating proliferating cell nuclear antigen (65). Vitamin D actually acts as an antiproliferative compound against small uterine fibroids by arresting cell growth (66). In the first randomized study, Arjeh et al (67) showed that addition of 50,000 IU vitamin D for 12 weeks inhibited growth of uterine fibroids whereas their volume increased in the placebo group (68).

6) Microbiome and uterine fibroids

Many intestinal microbes influence the physiological functions of the host. A number of bacteria in the intestinal flora can metabolize estrogen and they are referred to as the estrobolome. Their abundance correlates with systemic estrogen. For example, clostridium and pneumococcus exert the most significant effects on estrogen metabolism (69,70). Further and deeper analysis should help us understand whether gut microbiota could influence the risk of uterine fibroids Historically, female reproductive organs were considered uncontaminated, but in fact they are colonized by microorganisms. The human microbiome project reported that the uterine cavity harbors a unique microbiome (71,72) whose specific function remains unknown. So far, there is no evidence that the uterine microbiome plays a role in the pathogenesis of uterine fibroids. Evaluation of a possible role should be regarded as a critical aspect of future investigations.

4- Conclusions

The current review focuses on the pathogenesis of and risk factors for developing uterine fibroids. There is no doubt that a better understanding of the pathogenesis of leiomyomas could lead to more effective and comprehensive management of these risk factors, and thereby possibly prevent their development (9). We need to gather prospective data and conduct studies to compare alternative approaches and assess long-term outcomes with

respect to quality of life, recurrence of symptoms (bleeding, bulk symptoms), fertility, and even complications (73,74). Very recently, Vafaei et al (75) proposed an approach termed ESCAPE (evidence-based approach for secondary prevention) and recommended several strategies to prevent occurrence of uterine fibroids and their potential recurrence after myomectomy.

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281 Figure 1: Risk factors for uterine fibroid. These include age, race, early menarche, parity, obesity,
282 hypertension, smoking, caffeine, alcohol, Vitamine D, endocrine disruptors and genetic
283 alterations.

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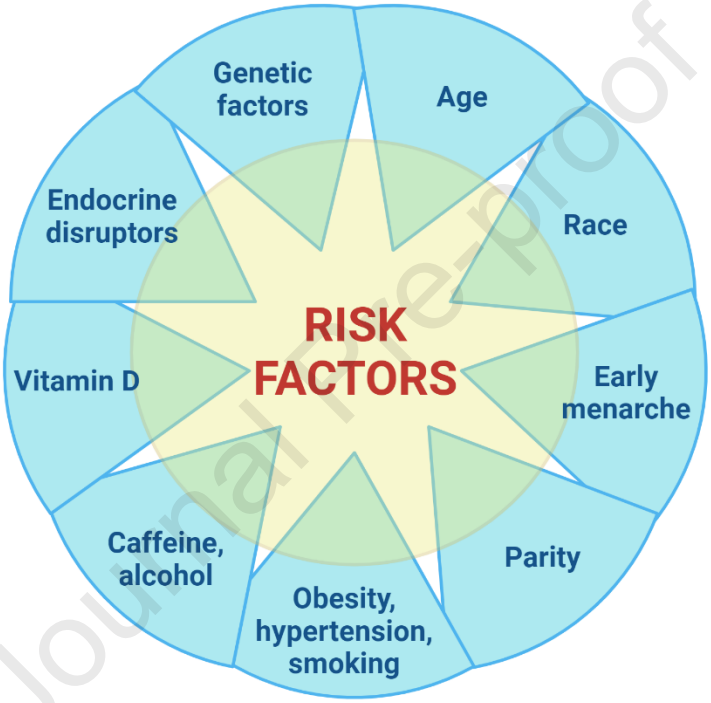

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REFERENCES

1. Critchley HOD, Babayev E, Bulun SE, Clark S, Garcia-Grau I, Gregersen PK, Kilcoyne A, Kim JJ, Lavender M, Marsh EE, Matteson KA, Maybin JA, Metz CN, Moreno I, Silk K, Sommer M, Simon C, Tariyal R, Taylor HS, Wagner GP, Griffith LG. Menstruation: science and society. Am J Obstet Gynecol. 2020;223(5):624-664.
2. Donnez J, Dolmans MM. Uterine fibroid management: From the present to the future. Hum. Reprod. Update 2016;22665–686.
3. Stewart EA. Clinical practice. Uterine fibroids. N. Engl. J. Med. 2015;372:1646–1655.
4. Lumsden MA, Hamoodi I, Gupta J, Hickey M. Fibroids: Diagnosis and management. BMJ 2015;51:h4887.
5. Okolo S. Incidence, aetiology and epidemiology of uterine fibroids. Best Pract. Res. Clin. Obstet. Gynaecol.2008;22:571–588.
6. Donnez J, Dolmans MM. Hormone therapy for intramural myoma-related infertility from ulipristal acetate to GnRH antagonist: A review. Reprod. Biomed. Online 2020;41:431–442.
7. Donnez J, Courtoy GE, Donnez O, Dolmans MM. Ulipristal acetate for the management of large uterine fibroids associated with heavy bleeding: A review. Reprod. Biomed. Online 2018;37:216–223.
8. Flynn M, Jamison M, Datta S, Myers E. Health care resource use for uterine fibroid tumors in the United States. Am J Obstet Gynecol. 2006;195:955-64.
9. Dolmans MM, Cacciottola L, Donnez J. Conservative Management of Uterine Fibroid-Related Heavy Menstrual Bleeding and Infertility: Time for a Deeper Mechanistic Understanding and an Individualized Approach. J Clin Med. 2021;10(19):4389.
10. Joseph DS, Malik M, Nurudeen S, Catherino WH. Myometrial cells undergo fibrotic transformation under the influence of transforming growth factor beta-3. Fertil Steril. 2010;93(5):1500-8.
11. Gross KL, Panhuysen CI, Kleinman MS, Goldhammer H, Jones ES, Nassery N, Stewart EA, Morton CC. Involvement of fumarate hydratase in nonsyndromic uterine leiomyomas: genetic linkage analysis and FISH studies. Genes Chromosomes Cancer. 2004;41(3):183-90.12.
12. Rogers R, Norian J, Malik M, Christman G, Abu-Asab M, Chen F, Korecki C, Iatridis J, Catherino WH, Tuan RS, Dhillon N, Leppert P, Segars JH. Mechanical homeostasis is altered in uterine leiomyoma. Am J Obstet Gynecol. 2008;198(4):474.e1-11.
13. Norian JM, Malik M, Parker CY, Joseph D, Leppert PC, Segars JH, Catherino WH. Transforming growth factor beta3 regulates the versican variants in the extracellular matrix-rich uterine leiomyomas. Reprod Sci. 2009;16(12):1153-64.
14. Ciarmela P, Islam MS, Reis FM, Gray PC, Bloise E, Petraglia F, Vale W, Castellucci M. Growth factors and myometrium: biological effects in uterine fibroid and possible clinical implications. Hum Reprod Update. 2011;17(6):772-90.
15. Bernacchioni C, Ciarmela P, Vannuzzi V, Greco S, Vannuccini S, Malentacchi F, Pellegrino P, Capezzuoli T, Sorbi F, Cencetti F, Bruni P, Donati C, Petraglia F. Sphingosine 1-phosphate signaling in uterine fibroids: implication in activin A pro-fibrotic effect. Fertil Steril. 2021;115(6):1576-1585.
16. Yang Q, Ciebiera M, Bariani MV, Ali M, Elkafas H, Boyer TG, Al-Hendy A. Comprehensive Review of Uterine Fibroids: Developmental Origin, Pathogenesis, and Treatment. Endocr Rev. 2022 Jul 13;43(4):678-719.
17. Afify SM, Seno M. Conversion of stem cells to cancer stem cells: undercurrent of cancer initiation. Cancers (Basel). 2019;11(3).

18. Mas A, Stone L, O'Connor PM, Yang Q, Kleven D, Simon C, Walker CL, Al-Hendy A. Developmental Exposure to Endocrine Disruptors Expands Murine Myometrial Stem Cell Compartment as a Prerequisite to Leiomyoma Tumorigenesis. Stem Cells. 2017;35(3):666-678.
19. Peddada SD, Laughlin SK, Miner K, Guyon JP, Haneke K, Vahdat HL, Semelka RC, Kowalik A, Armao D, Davis B, Baird DD. Growth of uterine leiomyomata among premenopausal blanck and white women. Proc Natl AcadSci USA 2008;105:19887–19892.
20. Petraglia F, Serour GI, Chapron C. The changing prevalence of infertility. Int J Gynaecol Obstet 2013;123:S4–S8.
21. Pavone D, Clemenza S, Sorbi F, Fambrini M, Petraglia F. Epidemiology and Risk Factors of Uterine Fibroids. Best Pract Res Clin Obstet Gynaecol. 2018;46:3-11.
22. Marshall LM, Spiegelman D, Barbieri RL, Goldman MB, Manson JE, Colditz GA, Willett WC, Hunter DJ. Variation in the incidence of uterine leiomyoma among premenopausal women by age and race. Obstet Gynecol. 1997;90(6):967-73.
23. Wise LA, Palmer JR, Harlow BL, Spiegelman D, Stewart EA, Adams-Campbell LL, Rosenberg L . Risk of uterine leiomyomata in relation to tobacco, alcohol and caffeine consumption in the Black Women's Health Study. Hum Reprod 2004;19:1746–1754.
24. Stewart EA, Nowak RA. Uterine Fibroids: Hiding in Plain Sight. Physiology (Bethesda). 2022;37(1):16-27.
25. Eltoukhi HM, Modi MN, Weston M, Armstrong AY, Stewart EA. The health disparities of uterine fibroid tumors for African American women: a public health issue. Am J Obstet Gynecol 2014;210:194–198.
26. Baird DD, Dunson DB. Why is parity protective for uterine fibroids. Epidemiology. 2003;14:247–250.
27. Wise LA, Palmer JR, Spiegelman D, Harlow BL, Stewart EA, Adams-Campbell LL, Rosenberg L. Influence of body size and body fat distribution on risk of uterine leiomyomata in U.S. black women. Epidemiology 2005;16:346–354.
28. Wise LA, Laughlin-Tommaso SK. Epidemiology of uterine fibroids: from menarche to menopause. Clin Obstet Gynecol 2016;59:2–24.
29. Malone LJ. Myomectomy: reccurence after removal of solitary and multiple myomas. Obstet Gynecol 1969;34:200–209.
30. Donnez J, Donnez O, Dolmans MM. With the advent of selective progesterone receptor modulators, what is the place of myoma surgery in current practice. Fertil Steril 2014. a;102:640–648.
31. Kim J, Sefton EC. The role of progesterone signaling in the pathogenesis of uterine leiomyoma. Mol Cell Endocrinol. 2012;358:223–231.
33. Islam MS, Protic O, Stortoni P, Grechi G, Lamanna P, Petraglia F, Castellucci M, Ciarmela P.. Complex networks of multiple factors in the pathogenesis of uterine leiomyoma. Fertil Steril 2013;100:178–193.
34. Donnez J, Vázquez F, Tomaszewski J, Nouri K, Bouchard P, Fauser BC, Barlow DH, Palacios S, Donnez O, Bestel E, Osterloh I, Loumaye E; PEARL III and PEARL III Extension Study Group. Long-term treatment of uterine fibroids with ulipristal acetate ☆. Fertil Steril. 2014;101(6):1565-73.e1-18.
35. Reis FM, Bloise E, Ortiga-Carvalho TM. Hormones and pathogenesis of uterine fibroids. Best Pract Res Clin Obstet Gynaecol. 2016;34:13-24.
36. Laughlin SK, Schroeder JC, Baird DD.. New directions in the epidemiology of uterine fibroids. Semin Reprod Med 2010;28:204–217.

37. Boynton-Jarrett R, Rich-Edwards J, Malspeis S, Missmer SA, Wright R. A prospective study of hypertension and risk of uterine leiomyomata. Am J Epidemiol. 2005;161(7):628-638.
38. Takeda T, Sakata M, Isobe A, Miyake A, Nishimoto F, Ota Y, Kamiura S, Kimura T. Relationship between metabolic syndrome and uterine leiomyomas: a case-control study. Gynecol Obstet Invest. 2008;66(1):14-7.
39. Heilier JF, Donnez J, Lison D. Organochlorines and endometriosis: a mini-review. Chemosphere. 2008;71(2):203-10.
40. Mehine M, Kaasinen E, Mäkinen N, Katainen R, Kämpjärvi K, Pitkänen E, Heinonen HR, Bützow R, Kilpivaara O, Kuosmanen A, Ristolainen H, Gentile M, Sjöberg J, Vahteristo P, Aaltonen LA. Characterization of uterine leiomyomas by whole-genome sequencing. N Engl J Med 369: 43–53, 2013.
41. Mittal P, Shin YH, Yatsenko SA, Castro CA, Surti U, Rajkovic A. Med12 gain-of-function mutation causes leiomyomas and genomic instability. J Clin Invest 125: 3280–3284, 2015.
42. Mehine M, Mäkinen N, Heinonen HR, Aaltonen LA, Vahteristo P. Genomics of uterine leiomyomas: insights from high-throughput sequencing. Fertil Steril 102: 621–629, 2014.
43. Mäkinen N, Vahteristo P, Kämpjärvi K, Arola J, Bützow R, Aaltonen LA. MED12 exon 2 mutations in histopathological uterine leiomyoma variants. Eur J Hum Genet. 2013;21(11):1300-1303.
44. Mäkinen N, Mehine M, Tolvanen J, Kaasinen E, Li Y, Lehtonen HJ, Gentile M, Yan J, Enge M, Taipale M, Aavikko M, Katainen R, Virolainen E, Böhling T, Koski TA, Launonen V, Sjöberg J, Taipale J, Vahteristo P, Aaltonen LA. MED12, the mediator complex subunit 12 gene, is mutated at high frequency in uterine leiomyomas. Science. 2011;334(6053):252-5.
45. Gross KL, Panhuysen CI, Kleinman MS, Goldhammer H, Jones ES, Nassery N, Stewart EA, Morton CC. Involvement of fumarate hydratase in nonsyndromic uterine leiomyomas: genetic linkage analysis and FISH studies. Genes Chromosomes Cancer. 2004;41(3):183-90.
46. He C, Nelson W, Li H, Xu YD, Dai XJ, Wang YX, Ding YB, Li YP, Li T. Frequency of MED12 Mutation in Relation to Tumor and Patient's Clinical Characteristics: a Meta-analysis. Reprod Sci. 2022;29(2):357-365.
47. Sadeghi S, Khorrami M, Amin-Beidokhti M, Abbasi M, Kamalian Z, Irani S, Omrani M, Azmoodeh O, Mirfakhraie R. The study of MED12 gene mutations in uterine leiomyomas from Iranian patients. Tumour Biol. 2016;37(2):1567-71.
48. Croce S, Chibon F. MED12 and uterine smooth muscle oncogenesis: state of the art and perspectives. Eur J Cancer. 2015;51(12):1603-1610.
49. Heinonen HR, Sarvilinna NS, Sjöberg J, Kämpjärvi K, Pitkänen E, Vahteristo P, Mäkinen N, Aaltonen LA. MED12 mutation frequency in unselected sporadic uterine leiomyomas. Fertil Steril. 2014;102(4):1137-42.
50. Markowski DN, Helmke BM, Bartnitzke S, Löning T, Bullerdiek J. Uterine fibroids: do we deal with more than one disease? Int J Gynecol Pathol. 2014;33(6):568-572.
51. Matsubara A, Sekine S, Yoshida M, Yoshida A, Taniguchi H, Kushima R, Tsuda H, Kanai Y. Prevalence of MED12 mutations in uterine and extrauterine smooth muscle tumours. Histopathology. 2013;62(4):657-61.
52. Pérot G, Croce S, Ribeiro A, Lagarde P, Velasco V, Neuville A, Coindre JM, Stoeckle E, Floquet A, MacGrogan G, Chibon F. MED12 alterations in both human benign and malignant uterine soft tissue tumors. PLoS One. 2012;7(6):e40015.
53. Zhang Q, Ubago J, Li L, Guo H, Liu Y, Qiang W, Kim JJ, Kong B, Wei JJ. Molecular analyses of 6 different types of uterine smooth muscle tumors: Emphasis in atypical leiomyoma. Cancer. 2014;120(20):3165-77.

54. Äyräväinen A, Pasanen A, Ahvenainen T, Heikkinen T, Pakarinen P, Härkki P, Vahteristo P. Systematic molecular and clinical analysis of uterine leiomyomas from fertile-aged women undergoing myomectomy. Hum Reprod. 2020;35(10):2237-2244.
55. Heinonen HR, Pasanen A, Heikinheimo O, Tanskanen T, Palin K, Tolvanen J, Vahteristo P, Sjöberg J, Pitkänen E, Bützow R, Mäkinen N, Aaltonen LA. Multiple clinical characteristics separate MED12-mutation-positive and -negative uterine leiomyomas. Sci Rep. 2017;7(1):1015.
56. Elkafas H, Qiwei Y, Al-Hendy A. Origin of uterine fibroids: conversion of myometrial stem cells to tumor-initiating cells. Semin Reprod Med. 2017;35(6):481-486.
57. Hall JM, Greco CW. Perturbation of nuclear hormone receptors by endocrine disrupting chemicals: mechanisms and pathological consequences of exposure. Cells. 2019;9(1).
58. Toporova L, Balaguer P. Nuclear receptors are the major targets of endocrine disrupting chemicals. Mol Cell Endocrinol. 2020;502:110665.
59. Mlodawska OW, Saini P, Parker JB, Wei JJ, Bulun SE, Simon MA, Chakravarti D. Epigenomic and enhancer dysregulation in uterine leiomyomas. Hum Reprod Update. 2022;28(4):518-547.
60. Cardozo ER, Foster R, Karmon AE, Lee AE, Gatune LW, Rueda BR, Styer AK. MicroRNA 21a-5p overexpression impacts mediators of extracellular matrix formation in uterine leiomyoma. Reprod Biol Endocrinol 16: 46, 2018.
61. Fitzgerald JB, Chennathukuzhi V, Koohestani F, Nowak RA, Christenson LK. Role of microRNA-21 and programmed cell death 4 in the pathogenesis of human uterine leiomyomas. Fertil Steril 98: 726–734.e2, 2012.
62. Qiang W, Liu Z, Serna VA, Druschitz SA, Liu Y, Espona-Fiedler M, Wei JJ, Kurita T. Down-regulation of miR-29b is essential for pathogenesis of uterine leiomyoma. Endocrinology 155: 663–669, 2014.
63. Pike JW, Christakos S. Biology and mechanisms of action of the vitamin D hormone. Endocrinol Metab Clin North Am. 2017;46(4):815-843.
64. Halder SK, Goodwin JS, Al-Hendy A. 1,25-Dihydroxyvitamin D3 reduces TGF-beta3-induced fibrosis-related gene expression in human uterine leiomyoma cells. J Clin Endocrinol Metab. 2011;96(4):E754-E762.
65. Sharan C, Halder SK, Thota C, Jaleel T, Nair S, Al-Hendy A. Vitamin D inhibits proliferation of human uterine leiomyoma cells via catechol-O-methyltransferase. Fertil Steril. 2011;95(1):247-253.
66. Ciavattini A, Delli Carpini G, Serri M, Vignini A, Sabbatinelli J, Tozzi A, Aggiusti A, Clemente N. Hypovitaminosis D and "small burden" uterine fibroids: Opportunity for a vitamin D supplementation. Medicine (Baltimore). 2016;95(52):e5698.
67. Arjeh S, Darsareh F, Asl ZA, Azizi Kutenaei M. Effect of oral consumption of vitamin D on uterine fibroids: a randomized clinical trial. Complement Ther Clin Pract. 2020;39:101159.
68. Combs A, Singh B, Nylander E, Islam MS, Nguyen HV, Parra E, Bello A, Segars J. A Systematic Review of Vitamin D and Fibroids: Pathophysiology, Prevention, and Treatment. Reprod Sci. 2023;30(4):1049-1064.
69. Wang W, Li Y, Wu Q, Pan X, He X, Ma X. High-throughput sequencing study of the effect of transabdominal hysterectomy on intestinal flora in patients with uterine fibroids. BMC Microbiol. 2020;20(1):98.
70. Flores R, Shi J, Fuhrman B, Xu X, Veenstra TD, Gail MH, Gajer P, Ravel J, Goedert JJ. Fecal microbial determinants of fecal and systemic estrogens and estrogen metabolites: a cross-sectional study. J Transl Med. 2012;10:253.

71. Moreno I, Franasiak JM. Endometrial microbiota-new player in town. Fertil Steril. 2017;108(1):32-39.
72. Pelzer E, Gomez-Arango LF, Barrett HL, Nitert MD. Review: maternal health and the placental microbiome. Placenta. 2017;54:30-37.
73. Al-Hendy A, Myers ER, Stewart E. Uterine fibroids: burden and unmet medical need. Semin Reprod Med. 2017;35(6):473-480.
74. Donnez J, Carmona F, Maitrot-Mantelet L, Dolmans MM, Chapron C. Uterine disorders and iron deficiency anemia. Fertil Steril. 2022;118(4):615-624.
75. Vafaei S, Ciebiera M, Omran MM, Ghasroldasht MM, Yang Q, Leake T, Wolfe R, Ali M, Al-Hendy A. Evidence-Based Approach for Secondary Prevention of Uterine Fibroids (The ESCAPE Approach). Int J Mol Sci. 2023;24(21):15972.