Human myometrium and leiomyomas express gonadotropin-releasing hormone 2 and gonadotropin-releasing hormone 2 receptor

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Objective: To determine the presence or absence of a second form of GnRH (GnRH2) and corresponding receptor (GnRHR2) in human uterine myometrium and leiomyomata.
Design: Evaluation of human leiomyoma and patient-matched myometrium of differential mRNA and protein expression of GnRH2 and GnRHR2.
Setting: University hospital.
Patient(s): Eight women undergoing medically indicated hysterectomy for symptomatic fibroids.
Intervention(s): Microarray analysis, reverse-transcriptase polymerase chain reaction (RT-PCR), real-time RT-PCR, and immunohistochemistry.
Main Outcome Measure(s): Expression of mRNA and protein in leiomyoma and patient-matched myometrium.
Result(s): Microarray analysis demonstrated expression, and we confirmed the findings by RT-PCR. Real-time RT-PCR demonstrated equivalent expression of the genes in leiomyoma compared with patient-matched myometrium (0.99-fold for GnRH2 and 1.28-fold for GnRHR2). Immunohistochemistry confirmed the expression of GnRH2 protein in both leiomyoma and myometrium.
Conclusion(s): A second form of GnRH and corresponding receptor exists in the fibroid and myometrium. We speculate that an autocrine loop exists. Our findings provide further evidence that GnRH agonists may interact directly with GnRH receptors present in uterine fibroids. (Fertil Steril® 2007;88:39–46. ©2007 by American Society for Reproductive Medicine.)

Key Words: GnRH2 and GnRHR2, microarray analysis, RT-PCR, real-time RT-PCR, Western blot, immunohistochemistry

Uterine leiomyomas (fibroids) are the most common benign tumors in reproductive-aged women (1). Approximately 25% to 50% of women have symptoms from fibroids severe enough to warrant clinical intervention (2). The actual prevalence of fibroids may be significantly higher; the evaluation of pathologic specimens identified fibroids in 77% of the hysterectomy samples (3). Symptoms from fibroids include infertility, menorrhagia, pelvic pressure, pelvic pain, miscarriage, preterm labor, and stillbirth. Such dramatic and widespread symptoms require effective therapeutic options. Therapeutic interventions for leiomyomas can be surgical or medical. Unfortunately, the recurrence of fibroids following surgical therapies short of a hysterectomy ranges between 25% and 44% (4, 5), with a 2% risk of a planned myomectomy resulting in a hysterectomy (6).

Medical treatment for fibroids includes GnRH agonists (GnRHa), which induces a systemic hypoestrogenic state (7, 8) that induces fibroid regression. GnRHa can reduce fibroid size by 30% (9). However, following a course of medical treatment with GnRHa, fibroids return to pretreatment size within 6 months (10). Although the current understanding of GnRHa action suggests that medical management of uterine fibroids by GnRHa relies upon establishing a hypoestrogenic state, alternative explanations have not been fully explored.

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Ectopic production of GnRH and GnRH receptor, referred to as GnRH1 and GnRHR1, respectively, have been identified in tissues outside the brain. These tissues include immune cell lines (11–13), breast tissue (14, 15), placenta (16), ovary (17), endometrium (18, 19), myometrium, and leiomyoma (20, 21). GnRH1 is also present in breast cancer (14, 15), ovarian cancer (22), and endometrial cancer (23, 24). With a half-life of 2–4 minutes, local expression suggests an autocrine or paracrine function for GnRH.

In addition, there is increasing evidence of a second GnRH and GnRHR (25, 26). This second GnRH (GnRH2) has been demonstrated in humans in several tissues (breast, ovary, placenta, and endometrium) (14–17, 22, 27), and is expressed at higher concentrations outside the brain in humans (25). Although GnRH1 and GnRHR1 are also identified outside the brain, the expression of GnRH2 and GnRHR2 is expressed at higher concentrations outside the brain, and may have a distinct function compared with GnRH1 and GnRHR1. In ovarian cancers, it was demonstrated that GnRH1 antagonist exerts an antiproliferative effect through GnRH2 receptors (28). As a result, GnRH could potentially exert a direct antiproliferative action on fibroids via GnRHR2 if these receptors are present on fibroid cells.

We hypothesized that GnRH2 and GnRHR2 were present in uterine leiomyomas and adjacent myometrium. Such expression provides an alternative mechanism for GnRHa regulation of uterine fibroids, and could provide novel therapies that avoid the side effects of systemic GnRHa exposure.

MATERIALS AND METHODS

Tissue Procurement

Informed consent was obtained from eight women undergoing a hysterectomy with the diagnosis of a symptomatic fibroid uterus at the National Naval Medical Center in Bethesda, Maryland. Enrollment was open to subjects with all ethnic backgrounds and of reproductive age with different gravidity and parity. Subjects who used various modalities of medical management for fibroids were also included. The investigational review board of the National Naval Medical Center, Uniformed Services University of Health Sciences, and the National Institutes of Health approved this study. At the time of hysterectomy, leiomyoma and myometrial tissue were obtained randomly from the center, middle, and peripheral regions from large leiomyomas, and in the case of small fibroids, the entire fibroid was used.

Leiomyoma and myometrial tissue that was intended for RNA isolation was immediately minced and placed in RNAlater (Ambion, Inc., Austin, TX). Tissue samples were processed immediately or were placed at 4°C overnight and then stored at –70°C. Tissue that was intended for immunohistochemistry was cut into cubes and were about 5 mm to a side, that is, 125 mm^3, and stored in phosphate-buffered 10% formalin.

RNA Isolation

Total RNA from leiomyomas and myometrial samples was isolated following a standard protocol used in our laboratory (29, 30). The total RNA integrity was confirmed and the concentration quantified by agarose gel electrophoresis and spectrophotometry.

Microarray Analysis

Samples of 10–15 μg of total RNA from leiomyoma and myometrium were provided to Capital Genomix (Rockville, MD) for microarray analysis with Affymetrix technology. Thirty-three thousand genes on the U-133 Affymetrix chip were screened. Total RNA was handled according to Affymetrix guidelines (31).

Hybridization of purified, labeled cRNA was performed as described previously (31). Hybridized chips were scanned with the Hewlett-Packard GeneArray Scanner (Palo Alto, CA). Data analysis was performed with GeneChip software.

Reverse-Transcriptase polymerase Chain Reaction

Primers were designed using LASERGENE Navigator so as to amplify a 90- to 150-bp section close to the 3′ end of the translated region (within 500 bp). The primers were generated on a 392 DNA/RNA Synthesizer (Applied Biosystems, Foster City, CA) and purified by high-performance liquid chromatography. Primers for GAPDH were included as an internal control. Leiomyomas and myometrial samples were then electrophoresed side by side at the same starting concentration on a 2% agarose gel stained with ethidium bromide. Stock concentrations of total RNA from leiomyoma and myometrium treated with DNAse were also used to exclude the possibility of amplifying genomic DNA. Tissue samples used for reverse transcriptase polymerase chain reaction (RT-PCR) experiments were unrelated to the tissue samples used for microarray analysis.

Real-Time RT-PCR

Total RNA from leiomyoma or myometrium was diluted and primers were designed. The GnRH2 and GnRHR2 probe was designed with Primer Express software, version 1.5, and constructed by Applied Biosystems. An 18S RNA control was used for standardization to use as an internal standard to scale the measurements. All experiments were performed in triplicate, and data analysis was performed with Sequence Detector, version 1.7 (Applied Biosystems). Mid-logarithmic points were compared for statistical analysis.

Immunohistochemistry

Rabbit anti-GnRH-II antibody specificity has been defined previously (32, 33). This antibody has been used to identify the second form of GnRH in mouse (34), rats (35), and human neuronal cell lines (36) using immunocytochemistry. To determine the presence of GnRH2 in human leiomyomas

TABLE 1

Patient characteristics.

Patient	Race	Age (y)	Gravid	Para	Prior GnRHa use	Prior OCP use
1	C	35	12	2	N	N
2	AA	43	0	0	N	N
3	AA	34	0	0	N	N
4	AA	39	2	1	N	N
5	C	37	0	0	N	Y
6	AA	37	1	1	N	N
7	AA	35	0	0	N	Y
8	AA	46	4	2	N	N

Note: AA = African American; C = Caucasian; GnRHa = gonadotropin-releasing hormone agonist; OCP = oral contraceptive pills.

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and adjacent myometrium, slides were then deparaffined and hydrated in xylene and graded ethanol solutions followed by deionized water. Following established protocols in our lab, slides were prepped and blocking solution was drained and diluted primary antisera directed at GnRH2 was used at a dilution of 1:2,000. For a negative control, leiomyomas and myometrium samples were incubated with blocking solution with nonimmune serum instead of primary and secondary antibody specific antisera, and cardiac muscle was used as a negative tissue control. For a positive control, hypothalamus was used.

Statistical Analyses

Microarray analysis was done in a genome-wide fashion as a discovery phase, and therefore statistical analysis was not performed. Real-time RT-PCR was used as a quantitative measure of gene expression. Differential expression was evaluated by paired Student's *t* tests. Normalization with 18S was used to be able to express levels of gene in fibroids and myometrium Ct values. All experiments were performed in triplicate and confidence intervals were calculated.

FIGURE 1

Cluster analysis of microarray studies. Far *left*, gross analysis demonstrated significant molecular differences between leiomyoma and patient-matched myometrium. Analysis of gene ontology through chromosome analysis demonstrated consistent alterations in GnRH-related gene clusters. GnRH-associated genes identified are listed on the *right*.

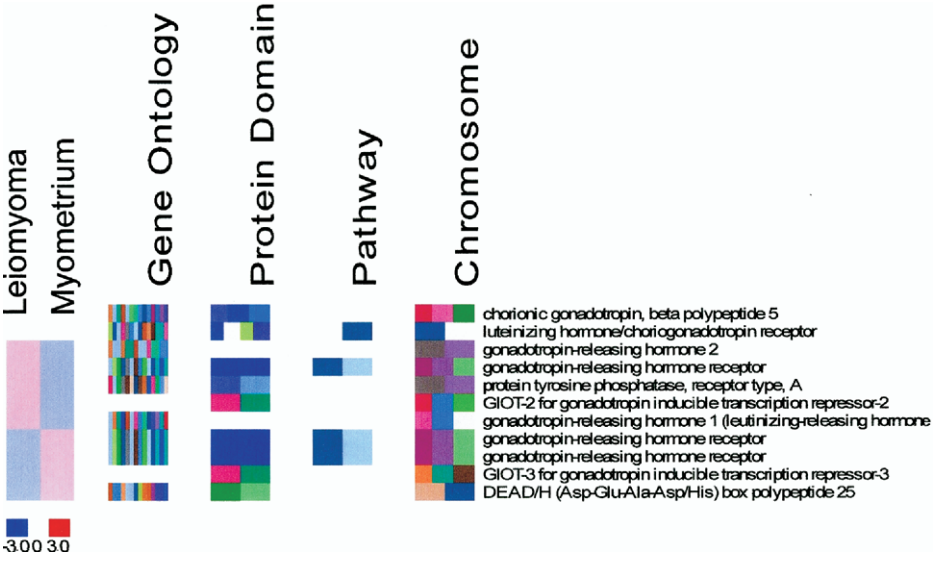

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TABLE 2

Cluster analysis of gonadotropin releasing hormone-related genes.

Name	Fold expression (M:F)
GnRH 1	0.44
GnRH 2	1.01
GnRH-RI	0.27
GnRH-RII	Undefined
βhCG	0.22
βLH	0.91
βFSH	1.08
LH receptor	1.00
FSH receptor	1.01
KISS-1	0.82
Calmodulin-1	1.79
Calmodulin-2	1.43
Calmodulin-3	0.81
Gonadotropin-regulated testicular RNA helicase	0.14
Gonadotropin-inducible transcription repressor	3.57

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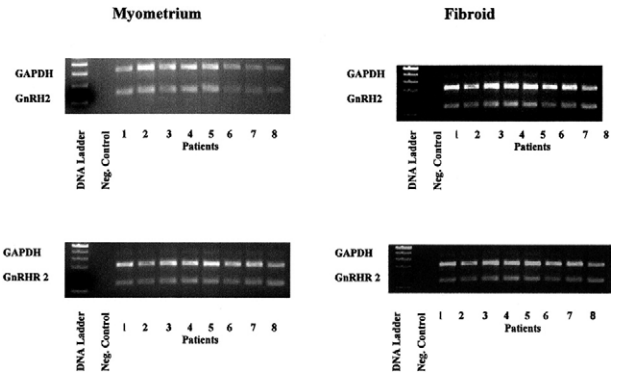
FIGURE 2

Reverse-transcriptase polymerase chain reaction (RT-PCR) for myometrium versus fibroid samples. Representative experiments are shown. Samples of myometrium and fibroid for eight patients were analyzed. Molecular marker (DNA molecular size standard, *lane 1*) and no template control (*lane 2*) were included on the *left*. GAPDH was used as an internal positive control.



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RESULTS

The demographic characteristics of the subjects evaluated in this study are presented in Table 1. There were six African Americans and two Caucasians enrolled in the study, and their ages ranged from 34 to 46 years. Subjects with different gravidity and parity were represented in this study. Patients who used oral contraceptive pills for the relief of symptoms related to fibroids were also included. No subjects had prior GnRHa treatment.

Microarray analysis screening was performed to determine if GnRH1 and GnRH2 with their corresponding receptors were identified in the myometrium and fibroid (Fig. 1, Table 2). These results demonstrated an equivalent expression of GnRH2 in fibroid compared with myometrium (0.99-fold). Microarray signal from labeled complementary template of GnRHR2 was too low to be reliably interpretable. This could have been because of specific mRNA degradation in those samples, or alternatively, the mRNA for GnRH2 was not present. It was of additional interest that GnRH1 and GnRHR1 were overexpressed in the fibroid compared with the myometrium (2.29- and 3.77-fold, respectively). Identification of GnRH1 and GnRHR1 expression in uterine fibroids has been described previously (20, 21).

We then performed confirmation studies on the expression of GnRH2 and GnRHR2 mRNA in leiomyomas and myometrium using multiplex RT-PCR of tissue samples from patients unrelated to those evaluated in the microarray analysis. Figure 2 demonstrated the expression of GnRH2 and GnRHR2 in both fibroid and myometrium from the patients. These results demonstrate the presence of GnRH2 and GnRHR2 mRNA in uterine fibroids from all patients tested. The internal control of GAPDH demonstrated that a similar

FIGURE 3

Representative real-time RT-PCR experiment. All eight patients were evaluated. The *x*-axis depicts the thermal cycle number while the *y*-axis represents luminescence (Δ*Rn*). The cycle at which exponential luminescence is achieved is dependent on the starting number of mRNAs for the gene in question. For GnRH2 and GnRHR2, the red lines represent fibroid and the blue lines represent myometrium. All experiments were performed in triplicate.

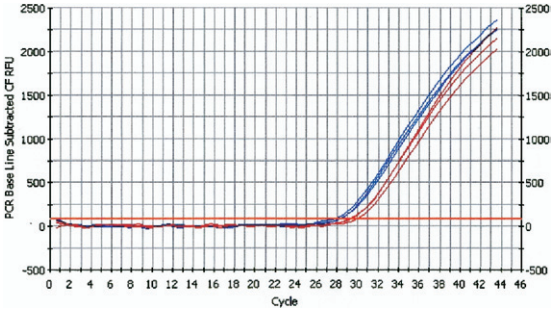

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TABLE 3

Comparison of differences between myometrium and fibroid for GnRH2.

Patient ID	Fold difference (M:F)	95% CI
1	0.20	(0.07, 0.33)[a]
2	0.74	(0.03, 1.47)
3	1.73	(0.48, 2.98)
4	0.49	(0.19, 0.79)[a]
5	0.86	(0.60, 1.12)
6	0.60	(0.30, 0.90)[a]
7	1.92	(0.20, 3.64)
8	1.38	(0.65, 2.11)
Mean	0.99	(0.47, 1.51)

Note: CI = confidence interval; ID = identification; M:F = myometrium versus fibroid.

[a] Indicates myometrium:leiomyoma pair that reached statistical significance.

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amount of material was loaded in each well. Expression was also verified using primers crossing introns and in specimens treated with DNAse (data not shown), which further confirmed the expression of mRNA for GnRH2 and GnRHR2 in fibroids and myometrium.

Although RT-PCR demonstrated the expression of both GnRH2 and GnRHR2, this method could not quantitate differential expression between fibroid and patient-matched

TABLE 4

Comparison of differences between myometrium and fibroid for GnRHR 2.

Patient ID	Fold difference (M:F)	95% CI
1	2.16	(1.64, 2.68)[a]
2	0.99	(0.01, 1.97)
3	1.44	(1.05, 1.83)[a]
4	1.49	(1.16, 1.82)[a]
5	1.36	(0.89, 1.83)
6	1.20	(0.60, 1.80)
7	1.11	(0.02, 2.00)
8	0.50	(0.01, 1.10)
Mean	1.28	(0.90, 1.66)

Note: CI = confidence interval; ID = identification; M:F = myometrium versus fibroid.

[a] Indicates myometrium:leiomyoma pair that reached statistical significance.

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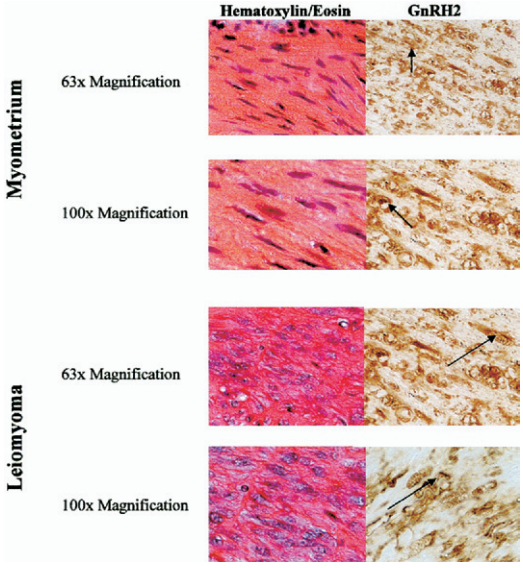
FIGURE 4

Light microscopy of myometrium and leiomyoma at low and high magnification. Hematoxylin and eosin staining on the *left*, and immunohistochemical stain for GnRH2 antibody on the *right*. The *arrow* indicates intracytoplasmic granules of concentrated GnRH2. GnRH2 antibody concentration was 1:2,000. Magnification = 63× (*above*) and 100× (*below*).



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myometrium (29). To quantitate relative mRNA expression, we optimized real-time RT PCR so as to evaluate a subtle difference in the degree of expression and provide quantitative information of relative expression. We found that the mean fold difference of GnRH2 in myometrium compared with fibroid was 0.99 (95% confidence interval [CI] [0.47, 1.51]), while the fold difference of GnRHR2 was 1.28 (95% CI [0.90, 1.66]; see Fig. 3, Table 3, and Table 4). These results demonstrate equivalent expression in fibroids and myometrium. Several individual fold differences for GnRH2 and GnRHR2 reached statistical significance, but overall the differences were not significant. All experiments were performed in triplicate.

The real-time RT-PCR data demonstrated a comparable mRNA expression of GnRH2 and GnRHR2 in leiomyomas compared with myometrium. To confirm that mRNA expression translated to protein expression, we determined the protein expression of GnRH2 (no antibody is commercially available for GnRHR2). Western blot analyses were attempted but were not reliably successful in detecting the 10 amino acid protein. Immunohistochemical studies were used to confirm the protein expression of GnRH2 in both leiomyoma and myometrium. Staining for GnRH2 was cytoplasmic in both tissues, appearing in cytoplasmic granules. There

was no significant staining in the cardiac muscle sample that served as a negative control, or in the leiomyomas and myometrium without primary antibody (Fig. 4).

DISCUSSION

Our study demonstrated that GnRH2 and GnRHR2 were expressed in the fibroid and myometrium at the mRNA level, and GnRH2 at the protein level. In addition, expression of GnRH2 and GnRHR2 were comparable in myometrium and fibroid in all patients evaluated. This observation expands upon the findings of White and colleagues (25), who have previously described the presence of GnRH2 and GnRHR2 in tissues other than the hypothalamus. This may provide an alternative explanation of the effects of GnRHa on regression of fibroids. Our study demonstrated equivalence regardless of the woman's age, times in cycle, and ethnicity.

This study demonstrated GnRH2 and GnRHR2 mRNA expression in fibroids and myometrium at a single point in time. What remains speculative is whether the expression of these genes is modulated by the activity of endogenous hormones and if this impacts the growth or regression of fibroids and if the expression of GnRH2 and GnRHR2 fluctuate throughout the menstrual cycle or throughout the reproductive years in the same patient. Studies are currently underway to address these questions by assessing a direct effect of GnRHa on GnRHR2 in human myometrium and fibroid cell lines.

Although GnRH and GnRHR are produced in the basal forebrain region, they are also located peripherally including reproductive and immune tissues throughout the body (18–20, 37–40). The presence of GnRH1 and GnRHR1 located throughout the reproductive system has led to the speculation of an autocrine and paracrine function (31, 41).

Our findings that a second form of GnRH, namely GnRH2, is expressed in myometrium, and fibroid tissue may give some insight to the apparent contradictory effect of GnRHa on certain reproductive tissues. An autocrine and paracrine function may explain the contradiction of why GnRH stimulates proliferation in some tissues and inhibits proliferation in others (11, 39, 38–43). The roles of these hormones may also be different depending on the hormonal milieu. The antiproliferative effects may even work through different GnRH receptors. In the case of some ovarian cancers, it has been demonstrated that GnRH1 antagonists exert an antiproliferative effect through GnRH2 receptors (37), and it is therefore possible that alternative modes of action may exist at the local level of the fibroid. Activation of the GnRH2 receptor pathway may have very distinct functions compared with activation of the GnRH1 receptor.

The GnRHa mediated fibroid regression is thought to occur by reducing the circulating levels of estrogen (44). The maximal GnRH-induced fibroid reduction occurs by 12 weeks, with no further decrease in size noted at 24 weeks (45). It is hypothesized that this is because of the decreased levels of circulating levels of estrogen. However, there are other mechanisms not directly related to the hypoestrogenic state. For example, GnRHa can reduce uterine blood flow (46), which may contribute toward the overall reduction in fibroid mass. These new findings suggest the possibility of a direct effect of GnRHa on the fibroid, which results in an antiproliferative effect via GnRH receptors expressed by leiomyocytes.

An autocrine or paracrine role for GnRH is supported by various explant and tissue culture experiments. Chegini and colleagues (20) were the first to demonstrate both the expression of GnRH1 and GnRHR1 expression in cultured leiomyoma cells, and have demonstrated that concentrations as low as 10^{-7} M of GnRH agonist can alter gene expression in tissue culture (47–49). Kobayashi and colleagues (21) confirmed GnRH1 and GnRHR1 expression in a leiomyoma explant culture system, and also identified phenotypic changes in leiomyoma cultures when treated with GnRHa. When primary cultures were treated with GnRHa, followed by microarray evaluation, there were distinct molecular changes that demonstrated a direct influence of GnRH1 on leiomyoma mRNA expression patterns (50). Finally, Kwon and colleagues (51) found that GnRH antagonist treatment increased mRNA and protein expression of GnRH receptor in cultured leiomyoma cells. Each of these studies demonstrated that local exposure can directly influence the leiomyocyte, and our findings provide support of a second GnRH pathway that could be involved in these changes.

Further study is warranted to determine if prior exposure to exogenous steroids (estrogens and progestins) as well as to GnRH agonist or antagonist may cause a shift in the relative expression of these genes in the uterus and fibroids. This understanding may lead to novel approaches of using GnRH agonist and antagonist at the specific peripheral receptor of interest instead of systemically. These results may help to increase our understanding of the possible implications of a local autocrine and paracrine relationship in symptomatic fibroid development.

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REFERENCES

1. Cramer DW. Epidemiology of myomas. Semin Reprod Endocrinol 1992;10:320–34.
2. Buttram V, Reiter R. Uterine leiomyomata: etiology, symptomatology and management. Fertil Steril 1981;36:433–45.
3. Cramer SF, Patel A. The frequency of uterine leiomyomas. Am J Clin Pathol 1990;94:435–8.
4. Fedele L, Parazzini F, Luchini L, Mezzopane R, Tozzi L, Villa L. Recurrence of fibroids after myomectomy: a transvaginal ultrasonographic study. Hum Reprod 1995;10:1795–6.
5. Fauconnier A, Chapron C, Babaki-Fard K, Dubuisson JB. Recurrence of leiomyomata after myomectomy. Hum Reprod Update 2000;6:595–602.

6. Iverson RE Jr, Chelmow D, Strohbehn K, Waldman L, Evantash EG. Relative morbidity of abdominal hysterectomy and myomectomy for management of uterine leiomyomas. Obstet Gynecol 1996;88:415–9.
7. Robboy SJ, Bentley RC, Butnor K, Anderson MC. Pathology and pathophysiology of uterine smooth-muscle tumors. Environ Health Perspect 2000;108:779–84.
8. Stewart EA. Uterine fibroids. Lancet 2001;357:293–8.
9. Felberbaum RE, Kupker W, Krapp M, Gehl B, Ludwig M, Diedrich K. Preoperative reduction of uterine fibroids in only 16 days by administration of a gonadotrophin-releasing hormone antagonist (Cetrotide). Reprod Biomed Online 2001;3:14–8
10. Vollenhoven BJ, Shekleton P, McDonald J, Healy DL. Clinical predictors for buserelin acetate treatment of uterine fibroids: a prospective study of 40 women. Fertil Steril 1990;54:1032–8.
11. Azad N, LaPaglia N, Kirsteins L, Uddin S, Steiner J, Williams DW, et al. Jurkat cell proliferative activity is increased by luteinizing hormone-releasing hormone. J Endocrinol 1997;153:241–9.
12. Chen HF, Jeung EB, Stephenson M, Leung PC. Human peripheral blood mononuclear cells express gonadotropin-releasing hormone (GnRH), GnRH receptor, and interleukin-2 receptor gamma-chain messenger ribonucleic acids that are regulated by GnRH in vitro. J Clin Endocrinol Metab 1999;84:743–50.
13. Chen A, Ganor Y, Rahimipour S, Ben-Aroya N, Koch Y, Levite M. The neuropeptides GnRH-II and GnRH-I are produced by human T cells and trigger laminin receptor gene expression, adhesion, chemotaxis and homing to specific organs. Nat Med 2002;8:1421–6.
14. Chen A, Kaganovsky E, Rahimipour S, Ben-Aroya N, Okon E, Koch Y. Two forms of gonadotropin-releasing hormone (GnRH) are expressed in human breast tissue and overexpressed in breast cancer: a putative mechanism for the antiproliferative effect of GnRH by down-regulation of acidic ribosomal phosphoproteins P1 and P2. Cancer Res 2002;62:1036–44.
15. Harris N, Dutlow C, Eidne K, Dong KW, Roberts J, Millar R. Gonadotropin-releasing hormone gene expression in MDA-MB-231 and ZR-75-1 breast carcinoma cell lines. Cancer Res 1991;51:2577–81.
16. Chou CS, Beristain AG, MacCalman CD, Leung PC. Cellular localization of gonadotropin-releasing hormone (GnRH) I and GnRH II in first-trimester human placenta and decidua. J Clin Endocrinol Metab 2004;89:1459–66.
17. Kang SK, Choi KC, Cheng KW, Nathwani PS, Auersperg N, Leung PC. Role of gonadotropin-releasing hormone as an autocrine growth factor in human ovarian surface epithelium. Endocrinology 2000;141:72–80.
18. Raga F, Casan EM, Kruessel JS, Wen Y, Huang HY, Nezhat C, et al. Quantitative gonadotropin-releasing hormone gene expression and immunohistochemical localization in human endometrium throughout the menstrual cycle. Biol Reprod 1998;59:661–9.
19. Dong KW, Marcelin K, Hsu MI, Chiang CM, Hoffman G, Roberts JL. Expression of gonadotropin-releasing hormone (GnRH) gene in human uterine endometrial tissue. Mol Hum Reprod 1998;4:893–8.
20. Chegini N, Rong H, Dou Q, Kipersztok S, Williams RS. Gonadotropin-releasing hormone (GnRH) and GnRH receptor gene expression in human myometrium and leiomyomata and the direct action of GnRH analogs on myometrial smooth muscle cells and interaction with ovarian steroids in vitro. J Clin Endocrinol Metab 1996;81:3215–21.
21. Kobayashi Y, Zhai YL, Iinuma M, Horiuchi A, Nikaido T, Fujii S. Effects of a GnRH analogue on human smooth muscle cells cultured from normal myometrial and from uterine leiomyomal tissues. Mol Hum Reprod 1997;3:91–9.
22. Grundker C, Gunthert AR, Millar RP, Emons G. Expression of gonadotropin-releasing hormone II (GnRH-II) receptor in human endometrial and ovarian cancer cells and effects of GnRH-II on tumor cell proliferation. J Clin Endocrinol Metab 2002;87:1427–30.
23. Irmer G, Burger C, Ortmann O, Schulz KD, Emons G. Expression of luteinizing hormone releasing hormone and its mRNA in human endometrial cancer cell lines. J Clin Endocrinol Metab 1994;79:916–9.
24. Chatzaki E, Bax CM, Eidne KA, Anderson L, Grudzinskas JG, Gallagher CJ. The expression of gonadotropin-releasing hormone and its receptor in endometrial cancer, and its relevance as an autocrine growth factor. Cancer Res 1996;56:2059–65.
25. White RB, Eisen JA, Kasten TL, Fernald RD. Second gene for gonadotropin-releasing hormone in humans. Proc Natl Acad Sci USA 1998;95:305–9.
26. Miyamoto K, Hasegawa Y, Nomura M, Igarashi M, Kangawa K, Matsuo H. Identification of the second gonadotropin-releasing hormone in chicken hypothalamus: evidence that gonadotropin secretion is probably controlled by two distinct gonadotropin-releasing hormone in avian species. Proc Natl Acad Sci USA 1984;81:3874–8.
27. Cheon KW, Lee HS, Parhar IS, Kang IS. Expression of the second isoform of gonadotrophin-releasing hormone (GnRH-II) in human endometrium throughout the menstrual cycle. Mol Hum Reprod 2001;7:447–52.
28. Grundker C, Emmons G. Role of gonadotropin-releasing hormone (GnRH) in ovarian cancer. Reprod Biol Endocrinol 2003;1:65–72.
29. Catherino WH, Prupas C, Tsibris JC, Leppert PC, Payson M, Nieman LK, et al. Strategy for elucidating differentially expressed genes in leiomyomata identified by microarray technology. Fertil Steril 2003;80:282–90.
30. Catherino WH, Leppert PC, Stenmark MH, Payson M, Potlog-Nahari C, Nieman LK, et al. Reduced dermatopontin expression is a molecular link between uterine leiomyomas and keloids. Genes Chrom Cancer 2004;40:204–17.
31. Lipshutz, RJ, Fodor SP, Gingeras TR, Lockhart DJ. High density synthetic oligonucleotide arrays. Nat Genet 1999;21:20–4.
32. Chen A, Yahalom D, Ben-Aroya N, Kaganovsky E, Okon E, Koch Y. A second isoform of gonadotropin-releasing hormone is present in the brain of human and rodents. FEBS Lett 1998:435:199–203.
33. Okuzawa K, Amano M, Kobayashi M, Aida K, Hanyu I, Hasegawa Y, et al. Differences in salmon GnRH and chicken GnRH-II contents in discrete brain areas of male and female rainbow trout according to age and stage of maturity. Gen Comp Endocrinol 1990:80:116–26.
34. Gestrin ED, White RB, Fernald RD. Second form of gonadotropin-releasing hormone in mouse: immunocytochemistry reveals hippocampal and periventricular distribution. FEBS Lett 1999:448:289–91.
35. Parhar IS, Soga T, Ogawa S, Ogawa S, Pfaff DW, Sakuma Y. Non-mammalian gonadotropin-releasing hormone molecules in the brain of promoter transgenic rats. Proc Natl Acad Sci 2005:102:5880–5.
36. Chen A, Yahalom D, Laskar-Levy O, Rahimipour S, Ben-Aroya N, Koch Y. Two isoforms of gonadotropin-releasing hormone are coexpressed in neuronal cell lines. Endocrinology 2001:142:830–7.
37. Grundker C, Gunthert AR, Westphalen S, Emons G. Biology of the GnRH system in human gynecological cancers. Eur J Endocrinol 2002;146:1–14.
38. Schally AV. LH-RH analogues: their impact on human reproduction and the control of tumorigenesis. Peptides 1999;20:1247–62.
39. Emons G, Schally AV. The use of luteinizing hormone releasing hormone agonists and antagonists in gynaecological cancers. Hum Reprod 1994;9:1364–1379.
40. Emons G, Ortmann O, Schulz KD, Schally AV. Growth inhibitory actions of analogues of luteinizing hormone releasing hormone on tumour cells. Trends Endocrinol Metab 1997;8:355–62.
41. Miller WR, Scott WN, Morris R, Fraser HM, Sharpe RM. Growth of human breast cancer cells inhibited by a luteinizing hormone-releasing hormone agonist. Nature 1985;313:231–3.
42. Batticane N, Morale MC, Gallo F, Farinella Z, Marchetti B. Luteinizing hormone-releasing hormone signaling at the lymphocyte involves stimulation of interleukin-2 receptor expression. Endocrinology 1991;129:277–86.
43. Marchetti B, Guarcello V, Morale MC, Bartoloni G, Raiti F, Palumbo G Jr, et al. Luteinizing hormone-releasing hormone (LHRH) agonist restoration of age-associated decline of thymus weight, thymic LHRH receptors, and thymocyte proliferative capacity. Endocrinology 1989;125:1037–45.
44. Friedman AJ, Harrison-Atlas D, Barbieri RL, Benacerraf B, Gleason R, Schiff I. A randomized, placebo controlled, double blind study evalu-

ating the efficacy of leuprolide acetate depot in the treatment of uterine leiomyomata. Fertil Steril 1989;51:251–6.
45. Lumsden MA, West CP, Baird DT. Goserelin therapy before surgery for uterine fibroids. Lancet 1987;1:36–7.
46. Matta WH, Stabile I, Shaw RW, Campbell S. Doppler assessment of uterine blood flow changes in patients with fibroids receiving the GnRH agonist buserelin. Fertil Steril 1988;49:1083–5.
47. Chegini N, Verala J, Luo X, Xu J, Williams RS. Gene expression profile of leiomyoma and myometrium and the effect of gonadotropin releasing hormone analogue therapy. J Soc Gynecol Invest 2003;10: 161–71.
48. Xu J, Luo X, Chegini N. Differential expression, regulation, and induction of Smads, transforming growth factor-beta signal transduction pathway in leiomyoma, and myometrial smooth muscle cells and alteration by gonadotropin-releasing hormone analog. J Clin Endocrinol Metab 2003;88:1350–61.
49. Ding L, Xu J, Luo X, Chegini N. Gonadotropin releasing hormone and transforming growth factor beta activate mitogen-activated protein kinase/extracellularly regulated kinase and differentially regulate fibronectin, type I collagen, and plasminogen activator inhibitor-1 expression in leiomyoma and myometrial smooth muscle cells. J Clin Endocrinol Metab 2004;89:5549–57.
50. Luo X, Ding L, Xu J, Williams RS, Chegini N. Leiomyoma and myometrial gene expression profiles and their responses to gonadotropin-releasing hormone analog therapy. Endocrinology 2005;146:1074–96.
51. Kwon JY, Park KH, Park YN, Cho NH. Effect of cetrorelix acetate on apoptosis and apoptosis regulatory factors in cultured uterine leiomyoma cells. Fertil Steril 2005;84:1526–8.