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ORIGINAL ARTICLE

# The 3´-untranslated *ApaI*-Insulin-like Growth Factor II Gene (*IGF2*) Polymorphism in Women with Uterine Leiomyomas

*Cláudia Aparecida Rainho,[1,3] MSc, PhD; Fabíola Encinas Rosa,[1,3] MSc; Anaglória Pontes,[2] MD, PhD; Silvia Regina Rogatto,[3] MSc, PhD*

*1 Department of Genetics, Biosciences Institute, UNESP – São Paulo State University - Botucatu, Brazil*

*2 Department of Gynecology and Obstetrics, School of Medicine, UNESP, São Paulo State University – Botucatu, Brazil*

*3 NeoGene Laboratory, Department of Urology, School of Medicine - UNESP, São Paulo State University - Botucatu, Brazil*

## ABSTRACT

OBJECTIVE: To explore a possible association between the rare allele A of the single nucleotide polymorphism in the 3´-untranslated region of the insulin-like growth factor II gene (*IGF2*), previously described as being associated with growth dysregulation, higher body mass index (BMI), and elevated risk of uterine leiomyomas. MATERIALS AND METHODS: A series of 144 women (72 clinically and histologically confirmed uterine leiomyomas and 72 without leiomyomas) was analyzed by a PCR-RFLP (Polimerase Chain Reaction – Restriction Fragment Length Polymorphism) based approach to detect a single nucleotide polymorphism in the 3´-untranslated region of the gene *IGF2*. Genotypic and allelic frequencies distributions in both groups were compared with weight, height, and BMI. RESULTS: No statistically significant differences in the genotype and allelic frequencies between patients and controls were observed. Similarly, the distribution of genotypes and weight, height, and BMI did not differ significantly between the two groups although the weight and BMI were lower in leiomyomas patients homozygous for allele A. CONCLUSIONS: The *ApaI* polymorphism of the *IGF2* gene does not produce different risk for leiomyoma development. Our data suggest that the deviations related in the genotypic frequencies for this polymorphism among women with uterine leiomyomas is not correlated with BMI and that previous associations were probably a result of chance statistical sampling present in a small studied group.

**Key words:** Insulin-like growth factor II gene. Single Nucleotide Polymorphism. Uterine Leiomyoma. Body Mass Index. Obesity.

## INTRODUCTION

Uterine leiomyomas are the most common benign neoplasia in women. They are an important cause of menorrhage and other forms of abnormal uterine bleeding. Besides, a spectrum of clinical symptoms such as abdominal pain, pressure sense, urinary incontinence, constipation, fetal wastage, and infertility are associated with their occurrence.[1] Clinical presentation depends on the size, location and number of the tumors. Based on these clinical signs, it was estimated to occur in approximately 30% of women in the thirty and forty decades of life. These tumors are rare in young and tend to regress after menopause. It is thought that leiomyomas rarely, if ever, progress to malignant leiomyosarcoma. Only those cases that are symptomatic are removed by myomectomy or hysterectomy, generally the only treatment options.[2] Although extensive research, in recent years, has advanced our understanding on molecular and cellular biology of these tumors, their etiology remains unclear .[3,4]

Initially, tumor growth may be dependent on promoting agents such as growth factors and hormones.[5] Growth factors and their receptors

*Correspondence*
*Silvia Regina Rogatto*
*NeoGene Laboratory, Department of Urology,*
*School of Medicine UNESP*
*18618-000 Botucatu, Brazil*
*Phone: 55 14 38116436 Fax: 55 14 38116271*
*E-mail: rogatto@fmb.unesp.br*

that are differentially regulated in leiomyomas or in the endometrium of leiomyomatous uteri are potential mediators of leiomyoma-related complications. One important pathway associated with growth of leiomyomas is the IGF-II receptor and cytokine. The insulin-like growth factor receptor (IGF-IR) and insulin-like growth factor II (IGF-II) mRNA are both overexpressed in leiomyomas.[4,6-8]

IGF-II, also known as somatomedin A, is a single chain polypeptide that shares amino acid sequence homology with insulin.[9] The gene encoding for this peptide (*IGF2* gene) was mapped on chromosome 11p15.5.[10] The regulation of *IGF2* gene expression and the function of this peptide in human cells is highly complex. This gene consists of nine exons and the mature peptide is encoded by exons 7, 8, and 9 and is transcribed from four different promoters (P1-P4).[11] Additionally, IGF-II signaling pathways involve two specific receptors and a family of six binding proteins as well as binding protein proteases, which in turn regulate IGF-II binding protein levels.[12] Interestingly, this gene is also regulated by genomic imprinting, a form of gene expression control in which a specific epigenetic modification specifies regulatory regions of this gene leading to differential expression of alleles depending on parental origin. In most normal tissues, the *IGF2* gene displays monoallelic paternal expression, with the maternal allele being silenced by imprinting.[13]

The IGF-II growth factor is known to support myoblasts differentiation[14] among other cell types. Gloudemans et al.[15] have observed a correlation between a polymorphism present on 3'-untranslated region of the *IGF2* gene and the occurrence of smooth muscle tumors. The authors have demonstrated that women homozygous for the absence of *AvaII* restriction site are more prone to develop leiomyoma than women who are heterozygous or homozygous for the presence of this site. Also, the development of a malignant smooth muscle tumor (leiomyosarcoma) in both men and women was correlated with homozygosity for the absence of the *AvaII* site. Furthermore, it was demonstrated that this *AvaII* site is also an *ApaI* site, previously described as linked sites.[16] More recently, these *ApaI-AvaII* restriction fragment length polymorphism (RFLP) have been correlated with risk of pathological body mass index (BMI). The positive association between this single nucleotide polymorphism in the 3'-unstraslated region and obesity in a sample of 1474 healthy men was first described by O'Dell et al:[17] mean BMI of rare AA homozygous (absence of *ApaI* or *p*resence of *AvaII* site) individuals was lower than of that common homozygous GG (presence of *ApaI* or absence of *AvaII* site), with heterozygous being intermediate.

In our study, we have compared the genotypic and allelic frequencies of the *ApaI-AvaII* RFLP on 3'-untranslated region of the *IGF2* gene between women with and without leiomyomas with the purpose of substantiating the frequencies already observed in leiomyoma patients. Furthermore, we have hypothesized that women with leiomyomas homozygous for the *IGF2* common allele G would also exhibited higher BMI when compared to women homozygous for the rare allele A, with or without leiomyomas.

## Materials and Methods

### Patients and Controls

We have studied 144 women undergoing surgical intervention in the gynecological unit of the Clinics Hospital of the School of Medicine of Botucatu, UNESP, São Paulo, Brazil. Informed consent to participate in the study was obtained from each woman according to the guidelines of the Hospital Ethical Committee.

We have classified the women into two groups: with leiomyomas or without leiomyomas (control group). The group with leiomyomas consisted of 72 patients with clinically and histologically confirmed diagnosis. A series of 72 women showing regular menstrual cycles, normal sonographic image, and undergoing laparoscopy due other indications and without history of previous uterine leiomyoma composed the clinical control group.

### Methodology of Genotyping

Genomic DNA obtained from peripheral blood by standard phenol / chloroform extraction was first amplified by polymerase chain reaction (PCR). The reaction mixture contained deoxynucleotide triphosphates (125 mM each), primers (0.2 mM each), 1.5 mM $MgCl_2$, 10 mM

Tris-HCl, pH 8.3, 50 mM KCl, and 1 U of Taq polymerase. The primers used were P2 (*sense*) 5'-CTTGGACTTTGAGTCAAATTG-3' and P3 (*antisense*) 5'-GGTCGTGCCAATTACATTTCA-3'.[18] A total of 35 cycles of PCR amplification were performed in a programmable thermal cycler GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California, USA). One cycle consisted of heat denaturation at 94ºC for 1 min, annealing at 55ºC for 2 min and extension at 72ºC for 3 min. The 292 bp product was then precipitated with ethanol, air-dried, and digested with 20U of *ApaI* endonuclease followed by electrophoresis. The undigested (292 bp) and digested *ApaI* alleles (231 bp and 61 bp) were named G allele and A allele, respectively (Figure 1).

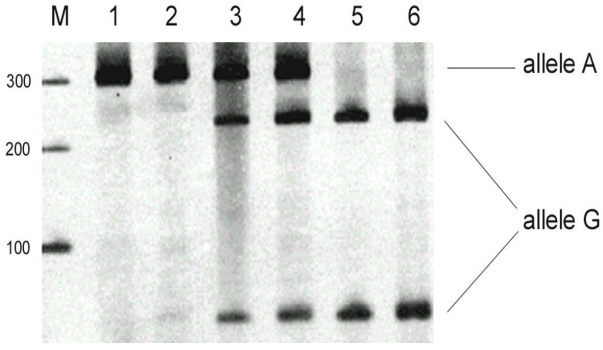


**Figure 1** - Representative electrophoresis pattern detected after PCR-RFLP analysis. The undigested (292 bp) and the digested *ApaI* alleles (231 bp and 61 bp) were named A allele and G allele, respectively (M = molecular weigth marker). The three possible genotypes were defined by the three distinct banding patterns: AA (lanes 1 and 2), AG (lanes 3 and 4) and GG (lanes 5 and 6)

## CLINICAL DATA

Mean weight, height and body mass index (BMI) were obtained for each woman. BMI was defined as the rate between weight in kilograms and the square of height in meters. This formula gives an estimate of the amount of fat stored in the body. Using this definition, values of BMI below 18 is considered underweight, between 18.5 and 24.9 normal, from 25 to 29.9 overweight, and greater than 30 obese.

## STATISTICAL ANALYSIS

The relationship between allelic and genotypic frequencies for each group was calculated according to the Hardy-Weinberg principle. Mean weight, height, and BMI for genotypes were compared by one-way analysis of variance (ANOVA). The statistical tests were performed using the statistical software package GraphPad InStat version 3.0, GraphPad Software, San Diego, California-USA (www.graphpad.com).

## RESULTS

To explore a possible association between the rare allele A of the single nucleotide polymorphism in the 3'-untranslated region of the insulin-like growth factor II gene (*IGF2*), previously described as being associated with growth dysregulation, higher BMI, and possible elevated risk of uterine leiomyomas, we have genotyped 144 women distributed into two groups according to the presence or absence of uterine leiomyomas. All 144 women were unrelated patients selected at the gynecological unit from our hospital. No correlations were detected between BMI and the *ApaI-IGF2*

**Table 1** - Distribution of *ApaI-IGF2* RFLP genotypes and means (±SD) weight, height and BMI (body mass index) parameters in uterine leiomyomas patients and controls

| Group | Age (years) | Weight (Kg) | Height (cm) | BMI (Kg/m$^2$) |
|---|---|---|---|---|
| Total of Women genotyped | 42.02±9.87 | 65.95±12.02 | 156.82±6.74 | 26.66±4.91 |
| Homozygous A / A | 45.69±12.85 | 64.25±10.43 | 159.50±6.52 | 25.29±3.76 |
| Heterozygous A / G | 43.85±10.43 | 66.33±12.27 | 158.40±6.01 | 26.44±4.94 |
| Homozygous G / G | 42.02±9.87 | 65.95±12.02 | 156.82±6.74 | 26.66±4.91 |
| With leiomyomas | 44.81±4.99 | 67.49±10.96 | 157.81±6.27 | 26.99±4.49 |
| Homozygous A / A | 45.57±3.41 | 61.24±7.45 | 160.71±6.82 | 23.80±3.40 |
| Heterozygous A / G | 44.73±4.87 | 69.53±8.93 | 158.96±4.92 | 27.53±3.55 |
| Homozygous G / G | 44.58±5.40 | 67.19±12.44 | 156.45±6.81 | 27.19±5.08 |
| Without leiomyomas (controls) | 41.35±11.66 | 65.92±12.89 | 156.49±6.70 | 26.88±5.08 |
| Homozygous A / A | 44.85±13.74 | 65.59±12.05 | 157.08±5.95 | 26.54±5.37 |
| Heterozygous A / G | 42.13±13.28 | 64.30±16.10 | 157.71±6.70 | 26.08±6.45 |
| Homozygous G / G | 39.54±9.52 | 67.08±11.01 | 156.11±7.11 | 27.51±4.51 |

genotypes in this group of 144 women (Table 1). The mean age was 44.81 (SD ±4.99) in the subgroup of patients with leiomyomas (n=72) and 41.35 (SD, ±11.66) in the control subgroup (n=72).

We have found 27 heterozygous AG, 7 homozygous AA and 38 homozygous GG cases in the group with leiomyomas. The frequency of A and G alleles calculated from these values was 0.28 and 0.72, respectively. In the subgroup without leiomyomas, we have detected 13 homozygous AA, 23 heterozygous AG and 36 homozygous GG cases. The frequency of A and G alleles for the control group was 0.34 and 0.66, respectively. The genotypes AA, AG, and GG for the *ApaI-IGF-2* polymorphism did not differ between patients with leiomyomas and the controls ($X^2$=2.174, p value 0.3372). A comparison between the genotypes AA and (AG+GG) have revealed no statistical differences between cases and controls (p=0.2277, Fisher's exact test). Comparing our genotypic and allelic frequencies with literature data, combined study group also show no statistical significance (Table 2).

We have calculated the BMI to determine whether there was correlation between different genotypes for *ApaI-IGF2* RFLP, body mass and the occurrence of leiomyomas. Mean BMI was 26.99 (SD, ± 4.49) for leiomyoma patients and 26.88 (SD, ± 5.08) for the control subgroup. The distribution of genotypes and weight, height, and BMI did not differ significantly between patients and controls (Table 1). Although mean BMI of the homozygous for allele A was lower in patients than mean BMI obtained from controls (23.80 and 26.54, respectively), this difference was not significant. Pathological obesity (BMI >30kg / $m^2$) was detected in 17 patients and in 17 controls (Table 3).

## DISCUSSION

One of the early observations that have improved our understanding on uterine leiomyomas biology was the identification of specific genetic mutations that occur in myometrial cells, leading to a progressive loss of growth regulation and to clonal expansion. Thus, leiomyomas arise from a complex process,

**Table 2** - Genotype distribution and allele frequency of the *ApaI-IGF2* polymorphism

| Group | Cases[a] | Genotypes | | | f(A) | f(G) | Ref. |
|---|---|---|---|---|---|---|---|
| | | A/A | A/G | G/G | | | |
| Without leiomyoma | 72 | 13 | 23 | 36 | 0.34 | 0.66 | This study |
| | 26 | 3 | 14 | 9 | 0.38 | 0.62 | 15 |
| | 66 | 4 | 28 | 34 | 0.27 | 0.71 | 26 |
| Total | 164 | 26 | 65 | 73 | 0.36 | 0.64 | |
| With leiomyoma(controls) | 72 | 7 | 27 | 38 | 0,28 | 0.72 | This study |
| | 11 | 0 | 4 | 7 | 0,18 | 0.82 | 15 |
| | 38 | 1 | 16 | 21 | 0,24 | 0.76 | 26 |
| | 20 | 5 | 9 | 6 | 0,48 | 0.52 | 27 |
| Total | 141 | 13 | 56 | 72 | 0,29 | 0.71 | |

(a) – number of cases
f(A) and f(G) – frequency of the *IGF2* A and G alleles.

**Table 3** - Distribution of body mass index (BMI) among genotypes

| Group | Cases[a] | BMI (Kg/$m^2$) | | | |
|---|---|---|---|---|---|
| | | <18 | 18.1-24.9 | 25.0-29.9 | >30 |
| With leiomyomas | | | | | |
| Homozygous A / A | 7 | 0 | 4 | 3 | 0 |
| Heterozygous A / G | 27 | 0 | 8 | 10 | 9 |
| Homozygous G / G | 38 | 1 | 12 | 17 | 8 |
| Without leiomyomas (controls) | | | | | |
| Homozygous A / A | 13 | 0 | 4 | 7 | 2 |
| Heterozygous A / G | 23 | 1 | 12 | 6 | 4 |
| Homozygous G / G | 36 | 0 | 13 | 12 | 11 |

(a) – number of cases.

which involves interactions between the effect of somatic mutations and myometrium microenvironment, since tumor growth may initially be dependent on growth factors, hormones and cytokines[19]. In such a model of multifactorial disease, it is believed that somatic mutation promoting dysregulation of some genes may predispose leiomyomas development.

Several human diseases (including cancer, osteoporosis, hypertension, diabetes) have been associated with allelic variants of susceptibility genes that can modify the risk of developing such conditions. The polymorphisms in the estrogen receptor *(ER)*[20,21], androgen receptor (*AR*)[22], steroid 17-alpha-hydroxylase (*CYP17*)[23], aromatase (*CYP19*)[23], and *IGF2*[15] genes has been investigated in uterine leiomyomas.

The IGF-II growth factor has proliferative activity in adult muscle is thought to play a central role in fetal growth and in the pathogenesis of several human tumors, including those arising from smooth muscle and from the breast[13-24]. Vafiadis et al[25], demonstrated that G alleles of the *ApaI-IGF2* polymorphism was associated with increased *IGF2* mRNA levels in leukocytes compared with the A alleles. It was hypothesized that this functional polymorphism could result in increased liver *IGF2* expression and secretion.

Gloudemans et al.[15] were pioneers in showing differences between allele frequencies in the *AvaI/ApaI*-RFLP in the *IGF2* gene among Dutch patients with uterine leiomyomas and a matched unaffected control group. It was found that homozygosity incidence for the absence of the *AvaII* site (allele G) at this locus was markedly higher in patients with leiomyosarcomas. Vu et al.[26] have demonstrated a similar correlation in American women with uterine smooth muscle tumors. Based on these data, it was suggested that women homozygous for allele G are more prone to develop leiomyomas. Our data for leiomyoma patients are not similar to those previously reported[15,26,27] and have shown a high frequency of individuals homozygous for G allele in the group of leiomyoma-free women. Furthermore, when analyzing literature and our data together, the combined study group has not shown statistical significance.

Some literature reports have investigated others risk factors for leiomyoma development. Descriptive studies suggest increased incidence with age, and black women appear to be disproportionately affected by this condition.[28] Epidemiologic studies among white patients undergoing hysterectomy for uterine leiomyomas have demonstrated a significant inverse association with parity, cigarette smoking and late age at last birth[29] and a significant positive association with obesity.[30,31] Samadi et al.[32] have demonstrated that leiomyomas are less frequent among women with a lower body mass index (BMI) who were current or long-time smokers.

An initial observation has reported a strong association between homozygosity for allele G of the *IGF2* gene and higher BMI in middle-aged males.[17, 33] In another independent study,[34] in which 500 healthy men and women were genotyped it was observed that individuals homozygous for the G allele did not exhibit significantly higher BMI or fat mass compared to AA individuals; however, individuals with the AA genotype exhibited higher fat mass. A contradictory finding was reported by Sun et al[35] in a group of 82 normal weight women (BMI<27Kg / $m^2$). These authors have shown that BMI was significantly higher in AA homozygous than in GG women. In our study, no significant differences were observed when we have compared the genotypic frequencies of *ApaI-IGF2* RFLP with weight, height and BMI between leiomyoma patients and controls. However, we have observed that between patients with leiomyomas heterozygous or homozygous for the G allele, the mean weight was 5.95-8.29kg higher than that observed for women with leiomyomas and homozygous for the A allele. The mean weight was similar for different genotypes in the control group. Although not statistically significant, in patients with leiomyomas the frequencies of RFLP were biased related to those observed in the group of women without lemiomyomas. These data could suggest that the presence of the G allele could be associated with a high BMI and this may indirectly influence the occurrence of uterine leiomyomas. However, lack of statistical significance provides no supports for the hypothesis that obese patients who are carriers of allele G constitute a group more prone to leiomyoma development. Similar results showing absence of significant correlation

between the *ApaI-IGF2* polymorphism and the BMI were related in a sample of 72 women with polycystic ovarian syndrome and 42 healthy controls.[36]

The putative association between the *ApaI-IGF2* RFLP and weight and BMI in women needs to be better substantiated. Our data are in disagreement with previous reports that demonstrate an association between this polymorphism and a higher risk for uterine leiomyomas development.

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## REFERENCES

1. Stewart EA, Nowak RA. Leiomyoma-related bleeding: a classic hypothesis updated for the molecular era. Hum Rep Update 1996;2:295-306.
2. Prayson RA, Hart WR. Pathologic considerations of uterine smooth muscle tumors. Obstet Clin North Am 1995;22:637-657.
3. Ligon AH, Morton CC. Leiomyomata: heritability and cytogenetic studies. Hum Rep Update 2001;7:8-14.
4. Tsibiris JCM, Segars J, Coppola D, Mane S, Wilbanks GD, O´Brien WF et al. Insights from gene arrays on the development and growth regulation of uterine leiomyomata. Fertil Steril 2002;78:114-21.
5. Rein MS, Nowak RA. Biology of uterine myomas and myometrium *in vitro.* Semin Rep Endocrinol 1992;10:310-9.
6. Hoopner JWM, Mosselman S, Roholl PJM. Expression of insulin-like growth factor I and II in smooth muscle tumors. EMBO J 1988;7:1379-85.
7. Boehm KD, Daimon M, Gorodeski I, Utian WH, Ilan J. Expression of the insulin-like and platelet-derived growth factor gene in human uterine tissues. Mol Rep Dev 1990;27: 93-101.
8. Vollenhoven BJ, Herington AC, Healy DL. Messenger ribonucleic acid expression of the insulin-like growth factors and their binding proteins in uterine fibroids and myometrium. J Clin Endocrinol Met 1993;76:1106-10.
9. Dull TJ, Gray A, Hayflick JR, Ullrich A. Insulin-like growth factor II precursor gene organization in relation to insulin gene family. Nature 1994;310:777-81.
10. Tricoli JV, Rall LB, Scott J, Bell GI, Shows TB. Localization of insulin-like growth factor genes to chromosomes 11 and 12. Nature 1994;310:784-6.
11. Schofield PN. Molecular biology of the insulin-like growth factors: gene structure and expression. Acta Pediatric Scand 1991;372:83-90.
12. D'Ercole AJ. Insulin-like growth factors and their receptors in growth. Endocrinol Metab Clin North Am 1996;25:573-90.
13. Toretsky JA, Helman LJ. Involvement of IGF-II in human cancers. J Endocrinol 1996;149:367-72.
14. Tollefsen SE, Sadow JL, Rotwein P. Coordinate expression of insulin-like growth factor II and its receptor during muscle differentiation. Proc Natl Acad Sci USA 1989;86:1543-7.
15. Gloudemans T, Pospiech I, Van der Ven LTM, Lips CJM, Den Otter W, Sussenbach JF. An AvaII restriction fragment length polymorphism in the insulin-like growth factor II gene and the occurence of smooth muscle tumors. Cancer Res 1993; 53:5754-8.
16. Schneid H, Girard F, Binoux M, Le Bouc Y. Ava II RFLP at the insulin-like growth factor II (IGF-II) locus on chromosome 11. Nucleic Acid Res 1989;17:466.
17. O´Dell SD, Miller GF, Cooper JA, Hindmarsh PC, Pringle PJ, Ford H, et al. ApaI polymorphism in insulin-like growth factor II (IGF2) gene and weight in middle-aged males. Int J Obes Relat Metab Disord 1997;21:822-5.
18. Ogawa O, Eccles MR, Szeto J, McNoe LA, Yun K, Maw MA, Smith PJ, et al. Relaxation of insulin-like growth factor II gene imprinting implicated in Wilms tumour. Nature 1993; 362:749-51.
19. Barbieri RL, Andersen J. Uterine leiomyomas: the somatic mutation theory. Sem Reprod Endocrinol 1992;10:301-9.
20. Massart F, Becherini L, Gennari L, Facchini V, Genazzani AR, Brandi ML. Genotype distribution of estrogen receptor-a gene polymorphism in Italian women with surgical uterine leiomyomas. Fertil Steril 2001;75:567-70.
21. Hsieh YY, Chang CC, Tsai FJ, Tsai HD, Yeh LS, Lin CC, et al. Estrogen receptor thymine-adenine dinucleotide repeat polymorphism is associated with susceptibility to leiomyoma. Fertil Steril 2003;79:96-9.
22. Fujimoto J, Hirose R, Sakaguchi H, Tamaya T. Expression of size-polymorphic androgen receptor gene in uterine leiomyoma according to the number of cytosine, adenine, and guanine repeats in androgen receptor alleles. Tumour Biol 2000;21:33-7.
23. Kado N, Kitawaki J, Obayashi H, Ishihara H, Koshiba H, Kusuki I, et al. Association of the CYP17 gene and CYP19 gene polymorphisms with risk of endometriosis in Japanese women. Hum Reprod 2002;17:897-902.
24. O'Dell SD, Day INM. Molecules in focus: Insulin-like growth factor II (IGF-II). Int J Biochem Cell Biol 1998;30:767-771.
25. Viafidis P, Bennett ST, Todd JA, Grabs R, Pylichronakos C. Divergence between genetic determinantes of IGF2 transcription levels in leukocytes and to IDDM2-encoded susceptibility type I Diabetes. J Clin Endocrinol Metab 1998;83:2933-9
26. Vu TH, Yballe C, Boonyanit S, Hoffman AR. Insulin-like growth factor II in uterine smooth-muscle tumors: maintenance of genomic imprinting in leiomyomata and loss of imprinting in leiomyosarcomata. J Clin Endocrinol Met 1995;80:1670-6.
27. Hashimoto K, Azuma C, Kamiura S, Koyama M, Nobunaga T, Tokugawa Y, et al. Maintenance of imprinitng of the insulin-like growth factor II gene (IGF2) and the small nuclear ribonucleoprotein polypeptide N gene (SNRPN) in the human uterus and leiomyoma. Gynecol Obstet Invest 1996;41:50-4.
28. Marshall LM, Spiegelman D, Barbieri RL, Goldman MB, Manson JE, Colditz GA, et al. Variation in the incidence of uterine leiomyoma among premenopausal women by age and race. Obstet Gynecol 1997;90:967-73.

29. Parazzini F, La Vecchia C, Negri E, Cecchetti G, Fedelle L. Epidemiologic characteristics of women with uterine fibroids: a case control study. Obstet Gynecol 1988;72:853-7.
30. Ross RK, Pike MC, Vessy MP, Yeates D, Casagrande JT. Risk factors for uterine fibroids: reduced risk associated with oral contraceptives. BMJ 986;293:359-62.
31. Settnes A, Jørgensen T, Lange AP. Hysterectomy in Danish women: weight-related factors, psychologic factors, and lyfe style variables. Obstet Gynecol 1996;88:99-105.
32. Samadi AR, Lee NC, Flanders D, Boring JR, Parris EB. Risk factors for self-reported uterine fibroids: a case-control study. Am J Publ Health 1996;86:858-62.
33. Gaunt TR, Cooper JA, Miller GJ, Day INM, O´Dell SD. Positive associations between single nucleotide polymorphism in the IGF2 gene region and body mass index in adult males. Hum Mol Genet 2001;10:1491-501.
34. Roth SM, Schrager MA, Metter EJ, Riechman SE, Fleg JL, Hurley BF, et al. IGF2 genotype and obesity in men and women across the adult age span. Int J Obes Metab Disord 2002;26:585-7.
35. Sun G, Chagnon YC, Gagnon J, Peruse L, Rao DC, Rivera MA, et al. ApaI polymorphism in IGF-II gene associated with higher BMI and fat mass (FM) in women from the Swedish Obese Subjects (SOS) and Quebec Family Study Cohorts. In: 8th International Congress Obesity Genetics and Molecular Basis of Obesity, Satellite Symposyum; 1998. [abstract number P18].
36. Sam Millan JL, Cortón M, Villuendas G, Sancho J, Peral B, Escobar-Morreale F. Association of the polycystic ovary syndrome with genomic variants related to insulin resistance, type 2 diabetes mellitus, and obesity. J Clin Endocrinol Metab 2004;89:2640-6.