Aromatase (*CYP19*) gene variants influence ovarian response to standard gonadotrophin stimulation

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Abstract

Purpose The association of cytochrome P450 aromatase gene *CYP19(TTTA)_n* polymorphism with ovarian response to FSH stimulation was explored.

Methods Three hundred women undergoing medically assisted reproduction and 300 women with at least one spontaneous pregnancy participated in the study. *CYP19(TTTA)_n* polymorphism was genotyped, while serum hormones were determined. During oocyte retrieval, the follicular size, the follicle and oocyte numbers were recorded.

Results Six *CYP19(TTTA)_n* alleles with 7 to 12 repeats were revealed. Women homozygous for long *CYP19(TTTA)_n*

alleles presented with lower serum FSH levels at the third day of the menstrual cycle ($p < 0.001$) and higher large follicle numbers ($p < 0.01$), compared to women homozygous for short *CYP19(TTTA)_n* alleles. The *CYP19(TTTA)₇* allele was associated with higher serum FSH levels ($p < 0.003$), with lower total follicle ($p < 0.02$) and large follicle numbers ($p < 0.03$), while *CYP19(TTTA)₇* allele-carriers presented more frequently with small follicles than *CYP19(TTTA)₇* allele-non carriers ($p < 0.01$).

Conclusions *CYP19* genetic variants were associated with ovarian reserve and response to standard gonadotrophin stimulation of women undergoing in vitro fertilization.

Capsule *CYP19* genetic variants are associated with ovarian reserve and response to standard gonadotrophin stimulation of women undergoing in vitro fertilization.

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Keywords Controlled ovarian stimulation · CYP19 · IVF · Ovarian response · Tubal infertility

Introduction

Cytochrome P450 aromatase is the key enzyme for the biosynthesis of estrogen from androgen. Aromatase is expressed in a wide range of tissues, including ovarian granulosa cells and luteal corpus, liver, muscles, breast, brain and testis [1]. Aromatase participates in human sexual development and reproduction by influencing estrogen bioavailability and androgen maintenance. High aromatase expression levels have been reported in pathological conditions, such as endometriosis [2] and breast cancer [3], whereas local aromatase activity has been associated with fibroids, adenomyosis and leiomyomas [4].

The crucial role of aromatase in the local regulation of ovarian functions has been revealed studying aromatase-deficient females. At birth, these females are characterized by hyperandrogenism and genital ambiguity, ranging from clitoral enlargement to complete labioscrotal fusion [5–7].

During childhood, abnormal functions of the LHRH-LH/FSH axis accompanied by polycystic ovaries [8, 9] and progressive delays in bone age have been reported in aromatase-deficient females [10]. Throughout puberty, these females show primary amenorrhea, multiple large ovarian cysts, breast development absence and further enlargement of clitoris [11, 12]. Finally, hypergonadotropic hypogonadism, congenital genital ambiguity, pubertal failure, virilisation, multicystic ovaries primary amenorrhea, breast development absence and infertility become more evident at adulthood [8, 11].

Cytochrome P450 aromatase is composed of two proteins, NADPH-cytochrome P450 reductase and cytochrome P450 aromatase. Human P450 aromatase is encoded by *CYP19* gene, which is composed by 9 translated exons encoding a unique protein of 55 kDa and 11 untranslated exons in the beginning of the gene [13]. Nineteen *CYP19* gene variants have been identified, four variants have substitutions in the coding exons, ten variants have substitutions in the untranslated exons, six variants have alterations in the 5' untranslated region and one in the 3' prime end [14].

The effects of *CYP19* gene variations on CYP19 activity have been investigated in a wide range of clinically important estrogen-dependent disorders. The most studied polymorphism is a short tetranucleotide tandem repeat (*TTTA*)_n in intron 4 of the *CYP19* gene, which has been involved in steroid hormone regulation. (*TTTA*)_n alleles have been associated with unexplained infertility and endometriosis [15], abdominal obesity [16], prenatal androgenisation leading to the development of polycystic ovary syndrome (PCOS) phenotype in adult life [17] and with enhanced vasomotor symptoms during the menopausal transition in women [18].

In the light of these observations, we sought to investigate whether (*TTTA*)_n alleles of the *CYP19* gene influence ovarian response of women undergoing standard gonadotrophin stimulation for in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), due to tubal and/or male factor infertility.

Materials and methods

Subjects

The study population was consisted of 300 women with tubal or male-factor infertility, referred to the IVF Unit of the Department of Obstetrics and Gynecology, Medical School of Ioannina, Greece, for IVF/ICSI application. Additionally, 300 women with at least one spontaneous pregnancy participated in the study as the control group. All women, aged 28 to 38 years, had normal BMI, normal menstrual cycles (28–30 days) and no signs of hyperandrogenism.

A detailed medical history was obtained from all subjects. Physical examination was performed. All women of the study population underwent a long GnRH agonist stimulation protocol as previously described [19]. Oocytes were retrieved by follicle aspiration by the transvaginal route under ultrasound guidance and the follicles were stratified into two groups according to their diameter (small follicles with diameter ≤12 mm and large follicles with diameter ≥18 mm).

Three embryos with the highest blastomere number and the best morphology were transferred the third day of each cycle. The remaining high-grade embryos were cryopreserved the same day. Pregnancy was diagnosed by quantitative β-hCG, 2 weeks after embryo transfer. Clinical pregnancy was confirmed by observing fetal cardiac activity on transvaginal ultrasound 4 weeks after a positive pregnancy test.

The Institutional Ethics Committee approved the study protocol in accordance to the Helsinki declaration and all participants gave informed consent.

Hormonal assays

Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E₂) were determined at the third day of the menstrual cycle by chemiluminescent microparticle immunoassay on an Abbott-ARCHITECT Immunoanalyser (Abbott Laboratories, Abbott Park, IL). The inter-assay coefficients of variation as indicated by manufactures were <4.6% for FSH, <4.1% for LH and <7.4% for E₂.

Genotype analysis

DNA was extracted from peripheral blood leukocytes. The *CYP19* (*TTTA*)_n repeat region was amplified by polymerase chain reaction (PCR), according to a protocol previously described [17, 20, 21]. The PCR products were separated by 10% polyacrylamide gel electrophoresis followed by silver staining, and the number of *TTTA* repeats in each allele was analysed by sequencing the appropriate PCR products [20]. Random sampling and sequencing were used for the assessment of quality control. All reactions were run in duplicates with positive, negative controls and blanks.

Statistical analysis

Statistical analysis was performed using the chi-square test. Normal distribution of continuous parameters was tested by Kolmogorov-Smirnov test. Differences in continuous parameters were assessed by using *t*-test for independent variables or the non-parametric Kruskal-Wallis test as appropriate. P-value of <0.05 was set as statistically significant. All results are reported as the mean±SD. All analyses used the SPSS

statistical package (version 14.0, SPSS Inc, Chicago, IL, USA).

Results

Clinical characteristics of the study population

During oocyte retrieval, the 300 women of the study population were presented with 14.1 ± 7.2 follicles, of which 6.9 ± 4.5 had large size while 4.5 ± 4.3 had small size, as well as with 8.5 ± 5.1 oocytes. The serum FSH, LH and E_2 of the study population at the third day of the menstrual cycle were 6.4 ± 2.8 mIU/ml, 5.1 ± 2.9 mIU/ml and 124 ± 35.9 pg/ml, respectively.

When we subdivided the study population in women with tubal infertility and in women with male factor infertility, the serum FSH, LH and E_2 levels at the third day of the menstrual cycle were significantly different between women with tubal and male factor infertility: 7 ± 1.7 mIU/ml vs. 6.1 ± 2.2 mIU/ml, $p < 0.004$; 5.6 ± 3.2 mIU/ml vs. 4.3 ± 2.2 mIU/ml, $p < 0.001$; 117.8 ± 41.7 pg/ml vs. 130.1 ± 31.5 pg/ml, $p < 0.01$, respectively.

Genotype analysis

The genotype analysis of the *CYP19(TTTA)_n* polymorphism revealed 6 *CYP19(TTTA)_n* alleles with 7 to 12 repeats. To analyse the association of this polymorphism with ovarian response to standard gonadotrophin stimulation, the *CYP19(TTTA)_n* alleles were divided into 2 subgroups using the *(TTTA)₉* allele as a cut-off point (based on the median number of *TTTA* repeats): short *CYP19* alleles with nine or fewer *TTTA* repeats and long *CYP19* alleles with more than nine *TTTA* repeats. The same cut-off allele has been used in previous studies exploring the distribution of the *CYP19(TTTA)_n* polymorphism [16, 20, 21].

The *CYP19* genotypic and allelic analysis did not show significant differences between the study population and the control group (data not shown). Additionally, when we subdivided the *CYP19* alleles in short and long, no

significant differences were observed between these groups (Table 1).

The genotype frequencies of the *CYP19(TTTA)_n* polymorphism were in Hardy-Weinberg equilibrium in the control group ($\chi^2 = 1.98$; P value > 0.05) and in the study population ($\chi^2 = 2.54$; P value > 0.05).

The association of the *CYP19(TTTA)_n* polymorphism with ovarian response to FSH stimulation

The simultaneous analysis of *CYP19(TTTA)_n* genotypes with serum FSH, LH and E_2 levels at the third day of the menstrual cycle, revealed a noteworthy association of long *CYP19(TTTA)_n* allele-homozygotes with lower FSH levels than short *CYP19(TTTA)_n* allele-homozygotes (6.1 ± 2.1 vs. 7.3 ± 1.8 mIU/ml, $p < 0.001$). Additionally, in women homozygous for long *CYP19(TTTA)_n* alleles, a higher number of large follicles was observed comparing with women homozygous for short *CYP19(TTTA)_n* alleles (7.9 ± 5.4 vs. 5.4 ± 4.1 , $p < 0.01$) (Fig. 1). However, no statistical significant trend was observed regarding the changes of serum FSH levels, total follicle and large follicle numbers in the range between 7 and 12 allelic repeats (data not shown). Furthermore, no significant association was found between *CYP19(TTTA)_n* genotypes and pregnancy rates.

When we analyzed the controlled ovarian stimulation (COS) outcome in combination with each *CYP19(TTTA)_n* allele, only the *CYP19(TTTA)₇* allele was significantly associated with serum FSH levels, follicle and large follicle numbers as well as with follicle size. Specifically, women with a *CYP19(TTTA)₇* allele in their genotype presented significantly higher serum FSH levels at the third day of the menstrual cycle compared to women without any *CYP19(TTTA)₇* allele in their genotype (7.5 ± 1.6 vs. 6.5 ± 2.1 mIU/ml, $p < 0.003$) (Fig. 2). In addition, in women without any *CYP19(TTTA)₇* allele in their genotype 14.6 ± 6.9 follicles and 7.6 ± 4.5 large follicles were produced, while in those with a *CYP19(TTTA)₇* allele in their genotype significantly fewer follicles and large follicles were produced (11.6 ± 4 and 5.7 ± 4.2 , $p < 0.02$ and $p < 0.03$, respectively) (Fig. 2). Finally, *CYP19(TTTA)₇* allele-carriers presented more frequently with

Table 1 The allelic distribution of the *CYP19(TTTA)_n* polymorphism in the study population group and in the control group

	Short <i>CYP19(TTTA)_n</i> Alleles	Long <i>CYP19(TTTA)_n</i> Alleles	p value
Study Population Group N (%)	338 (56.3)	262 (43.7)	ns
Control Group N (%)	350 (58.3)	250 (41.7)	

Data shown as number (N) and percentage (%)

Chi-square test analysis was used

Short *CYP19(TTTA)_n* alleles: *CYP19(TTTA)₇*, *CYP19(TTTA)₈*, *CYP19(TTTA)₉*

Long *CYP19(TTTA)_n* alleles: *CYP19(TTTA)₁₀*, *CYP19(TTTA)₁₁*, *CYP19(TTTA)₁₂*

Fig. 1 Association of *CYP19* (*TTTA*)_n genotypes with **a** serum FSH levels at the third day of the menstrual cycle ($p<0.001$) and **b** with large follicle number ($p<0.01$) in the study population. Data shown as mean value $\pm$ standard deviation. Short *CYP19* (*TTTA*)_n alleles: $n=7,8,9$. Long *CYP19*(*TTTA*)_n alleles: $n=10,11,12$

Fig. 2 Association of the *CYP19*(*TTTA*)₇ allele presence with **a** serum FSH levels at the third day of the menstrual cycle ($p<0.003$), **b** with follicle number ($p<0.02$) and **c** with large follicle number ($p<0.03$) in the study population. Data shown as mean value $\pm$ standard deviation

predominantly small follicles than *CYP19(TTTA)*₇ allele-non carriers (35.5% vs. 19%, $p < 0.01$). However, no significant association was found between *CYP19(TTTA)*₇ allele presence and pregnancy rates.

Discussion

Over the last decades many studies have focused on the crucial role of estrogen/androgen balance in female fertility and reproduction. Estrogen production and androgen bioavailability are regulated by cytochrome P450 aromatase enzyme. Aromatase is responsible for estrogen production in follicular granulosa cells using as substrate androgens, which are synthesized in theca cells. Each potential factor influencing aromatase activity, would also affect the levels of oestrogen and androgen and the regulation of their biosynthesis. In the present study, the genotype analysis of *CYP19(TTTA)*_n polymorphism in women undergoing ovarian stimulation for IVF or ICSI revealed six *CYP19(TTTA)*_n alleles. By subdividing the study population into subgroups according to the number of repeats, we found that women homozygous for long *CYP19(TTTA)*_n alleles presented with lower serum FSH levels at the third day of the menstrual cycle as well as with higher large follicle numbers, compared to women homozygous for short *CYP19(TTTA)*_n alleles. Whereas, when each *CYP19(TTTA)*_n allele was analyzed separately, significant associations of *CYP19(TTTA)*₇ allele with higher serum FSH levels, lower follicle and large follicle numbers as well as with small follicle size were observed in our study population.

The increased estrogen biosynthesis in endometrial tumours of (*TTTA*)₁₁/*(TTTA)*₁₁ and (*TTTA*)₁₁/*(TTTA)*₁₂ carriers [22] as well as the *CYP19(TTTA)*₁₁ allele presence in all members of a family with aromatase excess syndrome [23] have suggested a possible association of long *CYP19* alleles with an enhanced aromatase activity. On the other hand, PCOS women homozygous for short alleles had higher testosterone/estradiol ratios, higher testosterone levels and higher LH/FSH ratios compared to women homozygous for long alleles [17], probably due to their reduced aromatase activity. Regarding *CYP19(TTTA)*₇ allele, significant associations with lower estrone, estrone sulphate and estradiol concentrations have been reported [24, 25]. This allele has been also associated with lower sperm concentration and motility in normozoospermic and oligospermic men, due to potential impairment of the aromatase activity [21].

The hormone analysis of our study population revealed a noteworthy increase of FSH levels at the third day of the menstrual cycle in women with tubal infertility compared to women with male factor infertility. The high day-three FSH levels have been widely used as a marker of poor ovarian reserve and consequently of female infertility [26]. These

high levels of serum FSH at the third day of the menstrual cycle were associated with short *CYP19(TTTA)*_n alleles and mainly with the *CYP19(TTTA)*₇ allele in our study population, indicating a potential implication of *CYP19(TTTA)*_n polymorphism in serum FSH levels regulation. Taking into account the negative impact of short *CYP19(TTTA)*_n alleles on aromatase activity, we could explain the association of high serum FSH levels as a result of low aromatase efficiency. This finding is in accordance with previous studies examining infertile women suffering from aromatase deficiency. Specifically, inactivating mutations in the *CYP19* gene have been accompanied by markedly elevated FSH and circulating androgen concentrations as well as by low to undetectable estrone and estradiol levels [8, 9]. These FSH levels were suppressed after an estrogen replacement therapy [27], confirming the necessity of the aromatization of androgen to estrogen for the normal serum gonadotropin level and enhanced fertility preservation. The high levels of serum LH, FSH and androgens might reflect a central change in the activity of GnRH pulse generator and/or an effect at the pituitary level, probably induced by the increase of androgens and the aromatase deficiency [10]. The significance of aromatase activity in FSH level regulation has also been confirmed using aromatase inhibitors on an experimental basis. These drugs have been found to increase endogenous gonadotrophin levels by blockage of estrogen synthesis [28]. Consequently, we can conclude that *CYP19* gene variants participate in the serum FSH level adjustment but they do not constitute the only regulators, taking into account the presence of FSH isoforms with high or low bioactivities [29] as well as the *FSHR* genetic heterogeneity [30].

The second main finding of the current study is the association of *CYP19(TTTA)*_n alleles with COS outcome. The decreased follicle numbers, the lower amounts of large follicles as well as the smaller follicle sizes observed in women homozygous for short *CYP19(TTTA)*_n alleles or carriers of the *CYP19(TTTA)*₇ allele compared to women with other *CYP19* genotypes, result in a putative correlation of these alleles with a poorer response to COS. Our findings are in accordance with a previous study, in which short *CYP19(TTTA)*_n allele carriers presented with smaller ovaries, fewer antral follicles as well as with decreased ovarian FSH sensitivity in ovarian stimulation [15] and thus in need of increased gonadotropin administration during COS in order to achieve follicle numbers and sizes as high as those observed in women carriers of long *CYP19(TTTA)*_n alleles. Additionally, aromatase knockout mice presented with disrupted folliculogenesis, ovulation failure and elevated serum LH and FSH levels due to androgen/estrogen level imbalance [31]. Finally, the use of aromatase inhibitors during ovarian stimulation for intrauterine insemination has led to significantly lower amounts of large follicles compared to the control group [32], presumably due to reduced serum

estrogen levels and to increased ovarian follicular FSH receptor mRNA [33]. Taking into account the association of short *CYP19(TTTA)_n* alleles with reduced aromatase activity and lower serum estrogen levels, our results concerning follicular number and size, are indirectly supported.

Aromatase regulates estrogen production in follicular granulosa cells. Estrogens play a principal role in the development of follicles capable of responding to the appropriate hormonal stimulation to grow in size, produce a mature oocyte, and develop into a corpus luteum [34], whereas they regulate female reproduction at all levels of the hypothalamus-pituitary-ovary axis. Throughout follicular development, estrogen and androgen concentrations in follicular fluid undergo significant changes, as a result of modifications in the local concentrations of FSH and LH, in the follicular theca and granulosa cells and in response to the activity of enzymes involved in steroid biosynthesis, like aromatase [35]. The impaired aromatase activity would probably cause an androgen excess in utero, affecting negatively the anti-Müllerian hormone production by granulosa cells of growing pre-antral and small antral follicles, shown to be positively associated to ovarian follicle cohort size and reserve [36, 37]. Alterations in estrogen/androgen ratio, probably due to variations in aromatase activity, have also been found to be responsible for modifications in the expression of FSH receptor [38], affecting the total follicular number after a COS.

The results of the present study suggest that there is association of *CYP19(TTTA)_n* genotypes with serum FSH levels as well as with follicular numbers and sizes of women undergoing FSH stimulation for IVF/ICSI. Although our study is limited in size, is nevertheless indicative that the above genetic variants could be prognostic for the outcome of standard gonadotrophin stimulation. After the validation of our results in larger patient groups, *CYP19(TTTA)_n* genotype analysis could help in the selection of the proper controlled ovarian stimulation treatment in order to achieve a sufficient number of mature follicles and to increase the number of oocytes in poor responders.

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Conflicts of interest Authors have no conflicts of interest or any financial support to declare.

References

1. Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, Amarneh B, Ito Y, Fisher CR, Michael MD, Mendelson CR, Bulun SE. Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocr Rev*. 1994;15:342–55.
2. Zeitoun KM, Bulun SE. Aromatase: a key molecule in the pathophysiology of endometriosis and a therapeutic target. *Fertil Steril*. 1999;72:961–9.
3. Chen S, Itoh T, Wu K, Zhou D, Yang C. Transcriptional regulation of aromatase expression in human breast tissue. *J Steroid Biochem Mol Biol*. 2002;83:93–9.
4. Ishihara H, Kitawaki J, Kado N, Koshiba H, Fushiki S, Honjo H. Gonadotropin-releasing hormone agonist and danazol normalize aromatase cytochrome P450 expression in eutopic endometrium from women with endometriosis, adenomyosis, or leiomyomas. *Fertil Steril*. 2003;79:735–42.
5. Shozu M, Akasofu K, Harada T, Kubota Y. A new cause of female pseudohermaphroditism: placental aromatase deficiency. *J Clin Endocrinol Metabol*. 1991;72:560–6.
6. Ludwig M, Beck A, Wickert L, Bolkenius U, Tittel B, Hinkel K, Bidlingmaier F. Female pseudohermaphroditism associated with a novel homozygous G-to-A (V370-to-M) substitution in the P-450 aromatase gene. *J Pediatr Endocrinol Metab*. 1998;11:657–64.
7. Lin L, Ercan O, Raza J, Burren CP, Creighton SM, Auchus RJ, Dattani MT, Achermann JC. Variable phenotypes associated with aromatase (CYP19) insufficiency in humans. *J Clin Endocrinol Metabol*. 2007;92:982–90.
8. Conte FA, Grumbach MM, Ito Y, Fisher CR, Simpson ER. A syndrome of female pseudohermaphroditism, hypergonadotropic hypogonadism, and multicystic ovaries associated with missense mutations in the gene encoding aromatase (P450arom). *J Clin Endocrinol Metabol*. 1994;78:1287–92.
9. Mullis PE, Yoshimura N, Kuhlmann B, Lippuner K, Jaeger P, Harada H. Aromatase deficiency in a female who is compound heterozygote for two new point mutations in the P450arom gene: impact of estrogens on hypergonadotropic hypogonadism, multicystic ovaries, and bone densitometry in childhood. *J Clin Endocrinol Metabol*. 1997;82:1739–45.
10. Belgorosky A, Pepe C, Marino R, Guercio G, Saraco N, Vaiani E, Rivarola MA. Hypothalamic-pituitary-ovarian axis during infancy, early and late prepuberty in an aromatase-deficient girl who is a compound heterozygote for two new point mutations of the CYP19 gene. *J Clin Endocrinol Metabol*. 2003;88:5127–31.
11. Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. *J Clin Endocrinol Metabol*. 1995;80:3689–98.
12. Grumbach MM, Auchus RJ. Estrogen: consequences and implications of human mutations in synthesis and action. *J Clin Endocrinol Metabol*. 1999;84:4677–94.
13. Chow JD, Simpson ER, Boon WC. Alternative 5'-untranslated first exons of the mouse Cyp19A1 (aromatase) gene. *J Steroid Biochem Mol Biol*. 2009;115:115–25.
14. Lee SJ, Kim WY, Choi JY, Lee SS, Shin JG. Identification of CYP19A1 single-nucleotide polymorphisms and their haplotype distributions in a Korean population. *J Hum Genet*. 2010;55:189–93.
15. Altmäe S, Haller K, Peters M, Saare M, Hovatta O, Stavreus-Evers A, Velthut A, Karro H, Metspalu A, Salumets A. Aromatase gene (CYP19A1) variants, female infertility and ovarian stimulation outcome: a preliminary report. *Reprod Biomed Online*. 2009;18:651–7.
16. Baghaei F, Rosmond R, Westberg L, Hellstrand M, Eriksson E, Holm G, Björntorp P. The CYP19 gene and associations with androgens and abdominal obesity in premenopausal women. *Obes Res*. 2003;11:578–85.
17. Xita N, Lazaros L, Georgiou I, Tsatsoulis A. CYP19 gene: a genetic modifier of polycystic ovary syndrome phenotype. *Fertil Steril*. 2010;94:250–4.
18. Woods NF, Mitchell ES, Tao Y, Viernes HM, Stapleton PL, Farin FM. Polymorphisms in the estrogen synthesis and metabolism

- pathways and symptoms during the menopausal transition: observations from the Seattle Midlife Women's Health Study. *Menopause*. 2006;13:902–10.
19. Hatzi E, Bouba I, Galidi A, Lazaros L, Xita N, Sakaloglou P, Kolios G, Bairaktari E, Kaponis A, Zikopoulos K, Tsatsoulis A, Georgiou I. Association of serum and follicular SHBG levels and SHBG (TAAAA)n polymorphism with follicle size in women undergoing ovarian stimulation. *Gynecol Endocrinol*. 2011;27:27–32.
 20. Xita N, Georgiou I, Lazaros L, Psofaki V, Kolios G, Tsatsoulis A. The synergistic effect of sex hormone-binding globulin and aromatase genes on polycystic ovary syndrome phenotype. *Eur J Endocrin*. 2008;158:861–5.
 21. Lazaros L, Xita N, Kaponis A, Hatzi E, Plachouras N, Sofikitis N, Zikopoulos K, Georgiou I. The association of aromatase (CYP19) gene variants with sperm concentration and motility. *Asian J Androl*. 2011;13:292–7.
 22. Bernstein LM, Imyanitov EN, Kovalevskij AJ, Maximov SJ, Vasilyev DA, Buslov KG, Sokolenko AP, Iyevleva AG, Chekmariova EV, Thijssen JH. CYP17 and CYP19 genetic polymorphisms in endometrial cancer: association with intratumoral aromatase activity. *Cancer Lett*. 2004;207:191–6.
 23. Stratakis CA, Vottero A, Brodie A, Kirschner LS, DeAtkine D, Lu Q, Yue W, Mitsiades CS, Flor AW, Chrousos GP. The aromatase excess syndrome is associated with feminization of both sexes and autosomal dominant transmission of aberrant P450 aromatase gene transcription. *J Clin Endocrinol Metabol*. 1998;83:1348–57.
 24. Haiman CA, Hankinson SE, Spiegelman D, De Vivo I, Colditz GA, Willett WC, Speizer FE, Hunter DJ. A tetranucleotide repeat polymorphism in CYP19 and breast cancer risk. *Int J Cancer*. 2000;87:204–10.
 25. Tworoger SS, Chubak J, Aiello EJ, Ulrich CM, Atkinson C, Potter JD, Yasui Y, Stapleton PL, Lampe JW, Farin FM, Stanczyk FZ, McTiernan A. Association of CYP17, CYP19, CYP1B1, and COMT polymorphisms with serum and urinary sex hormone concentrations in postmenopausal women. *Cancer Epidemiol Biomarkers Prev*. 2004;13:94–101.
 26. Scott Jr RT, Hofmann GE. Prognostic assessment of ovarian reserve. *Fertil Steril*. 1995;63:1–11.
 27. Zirilli L, Rochira V, Diazzi C, Caffagni G, Carani C. Human models of aromatase deficiency. *J Steroid Biochem Mol Biol*. 2008;109:212–8.
 28. Mitwally MF, Casper RF. Use of an aromatase inhibitor for induction of ovulation in patients with an inadequate response to clomiphene citrate. *Fertil Steril*. 2001;75:305–9.
 29. Zambrano E, Zarinan T, Olivares A, Barrios-de-Tomasi J, Ulloa-Aguirre A. Receptor binding activity and in vitro biological activity of the human FSH charge isoforms as disclosed by heterologous and homologous assay systems: implications for the structure-function relationship of the FSH variants. *Endocrine*. 1999;10:113–21.
 30. de Koning CH, Benjamins T, Harms P, Homburg R, van Montfrans JM, Gromoll J, Simoni M, Lambalk CB. The distribution of FSH receptor isoforms is related to basal FSH levels in subfertile women with normal menstrual cycles. *Hum Reprod*. 2006;21:443–6.
 31. Britt KL, Drummond AE, Cox VA, Dyson M, Wreford NG, Jones ME, Simpson ER, Findlay JK. An age-related ovarian phenotype in mice with targeted disruption of the Cyp 19 (aromatase) gene. *Endocrinology*. 2000;141:2614–23.
 32. Bedaiwy MA, Mousa NA, Esfandiari N, Forman R, Casper RF. Follicular phase dynamics with combined aromatase inhibitor and follicle stimulating hormone treatment. *J Clin Endocrinol Metabol*. 2007;92:825–33.
 33. Weil S, Vendola K, Zhou J, Bondy CA. Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. *J Clin Endocrinol Metabol*. 1999;84:2951–6.
 34. Richards JS. Maturation of ovarian follicles: actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. *Physiol Rev*. 1980;60:51–89.
 35. Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocr Rev*. 1996;17:121–55.
 36. Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome NP, Visser JA, Kramer P, Fauser BC, Themmen AP. Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *Mol Hum Reprod*. 2004;10:77–83.
 37. Seifer DB, MacLaughlin DT. Mullerian Inhibiting Substance is an ovarian growth factor of emerging clinical significance. *Fertil Steril*. 2007;88:539–46.
 38. Luo W, Wiltbank MC. Distinct regulation by steroids of messenger RNAs for FSHR and CYP19A1 in bovine granulosa cells. *Biol Reprod*. 2006;75:217–25.