The ovarian response to standard gonadotrophin stimulation depends on *FSHR*, *SHBG* and *CYP19* gene synergism

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Abstract

Purpose Follicle stimulating hormone, sex hormone-binding globulin and cytochrome P450 aromatase play crucial roles in the regulation of mammalian reproduction. The synergistic effect of *FSHR* 307(T/A)/*FSHR* 680(N/S), *SHBG*(TAAAA)_n and *CYP19*(TTTA)_n genotypes on ovarian response to standard gonadotrophin stimulation of women undergoing medically assisted reproduction (IVF/ICSI) was explored.

Methods The study population consisted of 300 women under IVF/ICSI treatment and 300 women with at least with at least one successful child birth as controls. The polymorphisms were genotyped while the follicular size, the follicle and oocyte numbers were recorded during oocyte retrieval.

Results The genotype analysis, excluding heterozygotes for each particular polymorphism, revealed eight combined homozygotic *FSHR*/*SHBG*/*CYP19* genotypes. A gradual reduction in the number of follicles and oocytes from *FSHR* 307Thr/680Asn allele/long *SHBG* allele/long *CYP19* allele homozygotes to *FSHR* 307Ala/680Ser allele/short *SHBG*

allele/short *CYP19* allele homozygotes was observed (20.36±6.74 vs. 8.05±2.47, $p<0.008$ and 13±4.63 vs. 6.1±2.32, $p<0.02$, respectively).

Conclusions *FSHR*/*SHBG*/*CYP19* combined genotypes are associated with ovarian response to standard gonadotrophin stimulation of women undergoing medically assisted reproduction.

Keywords Controlled ovarian stimulation · *CYP19* · *FSHR* · IVF · *SHBG*

Introduction

During the last decades a growing body of evidence has accumulated regarding the regulation of ovarian function and consequently of female reproduction. A large number of molecules participate in this organ specific regulatory system, among which follicle stimulating hormone (FSH), sex hormone-binding globulin (SHBG) and cytochrome P450 aromatase (*CYP19*) play fundamental roles. FSH, which is produced and secreted by gonadotrope cells of the anterior pituitary gland, has been implicated in follicular growth and maturation, in granulosa cell proliferation and in estradiol/aromatase synthesis [1, 2]. FSH effects are mediated by FSH receptor (*FSHR*), a G-protein-coupled receptor expressed in granulosa cells [3]. On the other hand, the ovarian function is influenced by the granulosa lutein cell synthesized SHBG [4, 5], which constitutes the main transport protein of sex steroids to target tissues [6]. Finally, cytochrome P450 aromatase, also expressed in ovarian granulosa cells and luteal corpus [7], participates in human

Capsule The synergistic effect of *FSHR*, *SHBG* and *CYP19* genetic variants on ovarian response to standard gonadotrophin stimulation of women undergoing medically assisted reproduction (IVF/ICSI).

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reproduction by catalyzing the irreversible transformation of androgen to estrogen.

The effects of *FSHR*, *SHBG*, *CYP19* gene variants on the respective protein activity have been investigated in a wide range of clinically important disorders. The most frequent polymorphisms of the *FSHR* coding region are situated within exon 10. The first is located in the extracellular domain, changing codon 307 from threonine (Thr) to alanine (Ala), whereas the second is located in the intracellular domain, changing codon 680 from asparagine (Asn) to serine (Ser) [8]. Concerning *SHBG* gene, a $(TAAAA)_n$ pentanucleotide repeat polymorphism at the 5'-boundary of the gene promoter has been described, which has been shown to influence its transcriptional activity in vitro [9]. Furthermore, the most studied polymorphism of *CYP19* gene, which encodes human cytochrome P450 aromatase, is a short tetranucleotide tandem repeat $(TTTA)_n$ in intron 4 of the gene. This polymorphism has been involved in steroid hormone regulation [10].

Previous studies of our group have shown significant associations of *FSHR* 307(T/A)/*FSHR* 680(N/S), *SHBG* $(TAAAA)_n$ and *CYP19* $(TTTA)_n$ polymorphisms with the ovarian response to standard gonadotrophin stimulation [11–13]. The aim of the current study was to investigate the potential synergistic effect of the above polymorphisms on the follicular numbers and size, the oocyte numbers, the serum hormone levels and the pregnancy rate of women undergoing FSH stimulation for in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), due to tubal or male infertility factor.

Materials and methods

Subjects

300 Caucasian women with tubal or male-factor infertility, who referred to the IVF Unit of the Department of Obstetrics and Gynecology, Ioannina University Medical School, Greece, for IVF/ICSI, participated in the current study. Furthermore, 300 women with at least one successful child birth took part as a control group. All women had ages from 28 to 38 years, normal body mass index (BMI), normal menstrual cycles (28–30 days) and no signs of hyperandrogenism.

All subjects gave a detailed medical history, while physical examination was performed. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E_2) were determined at the third day of the menstrual cycle by chemiluminescent microparticle immunoassay using an Abbott-ARCHITECT Immunoanalyzer (Abbott Laboratories, Abbott Park, IL, USA). The intra-assay coefficients of variation were 3.5 % for LH, 4 % for FSH and 4.9 % for E_2 , while the inter-assay coefficients of variation were 8 % for

LH, 6.7 % for FSH and 6.4 % for E_2 . The SHBG concentration was measured using commercial ELISA kit (SHBG ELISA kit, BioSource, Nivelles, Belgium). The intra- and inter-assay coefficients of variation for SHBG were 3.8 % and 7.9 %, respectively. Samples were assayed in duplicate.

All women of the study population underwent a long GnRH agonist stimulation protocol as previously described [11, 14]. 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 respective 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 β -hCG quantification, 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.

Genotype analysis

DNA was extracted from peripheral blood leukocytes. *FSHR* 307 and *FSHR* 680 polymorphisms amplification was accomplished with polymerase chain reaction (PCR) using two primer pairs and PCR thermal cyclings previously described [12]. *FSHR* 307 PCR products were subsequently digested with restriction endonuclease *Bsu36BI*, while *FSHR* 680 PCR products with *BsrI*. Nucleotide change T>A at position 307 introduces a recognition site for *Bsu36BI* underlining the presence of Ala. The resulting genotypes were characterized as *TT*(307Thr/Thr), *TA*(307Thr/Ala), *AA*(307Ala/Ala). Change N>S at position 680 introduces a recognition site for *BsrI* underlining the presence of Ser. The resulting genotypes were characterized as *NN*(680Asn/Asn), *NS*(680Asn/Ser) and *SS*(680Ser/Ser). Enzyme cleavage products were separated by 3 % agarose gel electrophoresis and visualized by exposure to ultraviolet light after ethidium bromide staining. All reactions were run in duplicates with negative and positive controls and blanks.

The *SHBG* $(TAAAA)_n$ and *CYP19* $(TTTA)_n$ repeat regions were amplified by PCR using primer pairs and thermal cyclings previously described [11, 13]. The PCR products were separated by 10 % polyacrylamide gel electrophoresis and visualized after silver staining. Genotyping error was avoided in our analysis because all PCR products were run in duplicates with short and long genotype controls and other intermediate samples of known $(TAAAA)_n$ and $(TTTA)_n$ repeats, respectively. The controls were previously analyzed

by sequencing. In case of discrepant results, a second round of duplicates was run. Also random sampling and sequencing were done for quality control purposes.

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 between groups and genotypes were assessed with *t*-test and confirmed with the non-parametric Kruskal–Wallis test. *P*-value of <0.05 was set as statistically significant. All results are reported as the mean±SD. All analyzes used the SPSS statistical package (version 14.0, SPSS Inc, Chicago, IL, USA).

Results

Clinical characteristics

The clinical characteristic of the study population and the control group are presented in Table 1. No significant differences were observed in the age, BMI and serum FSH, LH, E₂ and SHBG levels between the two groups using the *t*-test.

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.

Genotype analysis

The *FSHR* 307(T/A)/*FSHR* 680(N/S) diplotype analysis revealed three diplotypes: *Thr*307*Thr*/*Asn*680*Asn*, *Thr*307*Ala*/*Asn*680*Ser*, *Ala*307*Ala*/*Ser*680*Ser*, proving the linkage of the polymorphisms [8]. No significant differences in *FSHR* 307(T/A)/*FSHR* 680(N/S) diplotype frequencies were observed between the study population and the control group (data not shown). These diplotypes were in Hardy-Weinberg

equilibrium in both study population ($\chi^2=0.05$; $p>0.05$) and control group ($\chi^2=0.13$; $p>0.05$).

The genotype analysis of the *SHBG*(TAAAA)_n polymorphism resulted in 5 (TAAAA)_n alleles with 6–11 repeats. For the analysis of the effect of this polymorphism, the study population was subdivided in subgroups using as cut off the (TAAAA)₈ allele of the SHBG gene: women with short *SHBG* genotypes (both alleles with ≤8 TAAAA repeats) and women with long *SHBG* genotypes (both alleles with >8 TAAAA repeats). The same cut-off allele has been used in previous studies exploring the distribution of the *SHBG*(TAAAA)_n polymorphism [13, 15]. The *SHBG*(TAAAA)_n genotype frequencies did not present significant differences between the study population and the control group ($p>0.05$).

Finally, the genotype analysis of the *CYP19*(TTTA)_n polymorphism revealed 6 *CYP19*(TTTA)_n alleles with 7 to 12 repeats. To analyze 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 [12, 16, 17]. The genotype frequencies of the *CYP19*(TTTA)_n polymorphism did not show significant differences between the two groups ($p>0.05$).

The combined *FSHR*/*SHBG*/*CYP19* genotype analysis resulted in eight homozygotic genotypes, after excluding *FSHR* 307(T/A)/*FSHR* 680(N/S), *SHBG*(TAAAA)_n and *CYP19*(TTTA)_n heterozygotes so as to explore the true allelic impact on ovarian response to standard gonadotrophin stimulation. The total number of the homozygotic combined genotypes in the study population was 113 while in the control group 104 (Table 2). The combined *FSHR*/*SHBG*/*CYP19* genotype frequencies did not differ significantly between the study population and the control group.

The association of the combined *FSHR*/*SHBG*/*CYP19* genotypes with ovarian response to standard gonadotrophin stimulation

The analyzed genotypes were associated with the data collection during previous IVF-ICSI cycles. A gradual reduction in the number of follicles from *FSHR* 307*Thr*/680*Asn* allele/long *SHBG* allele/long *CYP19* allele homozygotes to *FSHR*307*Ala*/680*Ser* allele/short *SHBG* allele/short *CYP19* allele homozygotes was detected (20.36±6.74 vs. 8.05±2.47, $p<0.008$) (Fig. 1a). Similar results were found for oocytes. In specific, from *FSHR* 307*Thr*/680*Asn* allele/long *SHBG* allele/long *CYP19* allele homozygotes to *FSHR*307*Ala*/680*Ser* allele/short *SHBG* allele/short *CYP19* allele homozygotes, a gradual decrease in oocyte number was observed (13±4.63 vs. 6.1±2.32,

Table 1 Characteristics of the study population and the control group

	Study population	Control group	<i>p</i> -value
Number of patients	300	300	
Age of patients (years)	29.2±7.3	28.9±7.7	ns
Body mass index (kg/m ²)	22.8±3.2	23.1±3.8	ns
FSH (mIU/ml)	6.4±2.8	6.8±2.2	ns
LH (mIU/ml)	5.1±2.9	4.6±2.8	ns
E ₂ (pg/ml)	124±35.9	133±40.5	ns
SHBG (nmol/l)	69.1±34.5	72.2±35.1	ns

Data shown as mean value±standard deviation

ns non significant

Table 2 The distribution of the combined homozygotic *FSHR/SHBG/CYP19* genotypes in the study population and in the control group

Combined homozygotic <i>FSHR/SHBG/CYP19</i> genotypes		Study population group N	Control group N
CG1	<i>FSHR</i> 307Thr/680Asn allele/long <i>SHBG</i> allele/long <i>CYP19</i> allele homozygotes	12	10
CG2	<i>FSHR</i> 307Thr/680Asn allele/short <i>SHBG</i> allele/long <i>CYP19</i> allele homozygotes	11	11
CG3	<i>FSHR</i> 307Thr/680Asn allele/long <i>SHBG</i> allele/short <i>CYP19</i> allele homozygotes	12	11
CG4	<i>FSHR</i> 307Thr/680Asn allele/short <i>SHBG</i> allele/short <i>CYP19</i> allele homozygotes	14	13
CG5	<i>FSHR</i> 307Ala/680Ser allele/long <i>SHBG</i> allele/long <i>CYP19</i> allele homozygotes	13	14
CG6	<i>FSHR</i> 307Ala/680Ser allele/short <i>SHBG</i> allele/long <i>CYP19</i> allele homozygotes	13	12
CG7	<i>FSHR</i> 307Ala/680Ser allele/long <i>SHBG</i> allele/short <i>CYP19</i> allele homozygotes	15	13
CG8	<i>FSHR</i> 307Ala/680Ser allele/short <i>SHBG</i> allele/short <i>CYP19</i> allele homozygotes	23	20

Data shown as number (N)

$p < 0.02$) (Fig. 1b). The presence of *FSHR* 307Thr/680Asn, long *SHBG* and long *CYP19* alleles was associated with higher follicle and oocytes numbers, while the presence of *FSHR*307Ala/680Ser, short *SHBG* and short *CYP19* alleles was associated with lower follicle and oocytes numbers.

However, no statistical significant association was observed between the combined *FSHR/SHBG/CYP19* genotypes and the follicular size or the pregnancy rates. Finally, the association analysis of the combined *FSHR/SHBG/CYP19* genotypes with serum FSH, LH, E₂ and SHBG levels at the third day of the menstrual cycle, revealed no significant correlations.

Discussion

Intrafollicular steroids and their metabolism are crucial for the response and fate of individual follicles [18, 19]. The intrafollicular estrogen production takes place in follicular granulosa cells using as substrate androgens, which are synthesized in theca cells under the catalytic action of cytochrome P450 aromatase [7]. However, estrogen and aromatase production depends on granulosa cell synthesized FSHR [2, 3], the mediator of FSH effects. On the other hand, the transport of intraovarian/intrafollicular sex steroids to target cells/tissues

Fig. 1 Association of the combined homozygotic *FSHR/SHBG/CYP19* genotypes with **a** the follicle numbers ($p < 0.008$) and **b** the oocyte numbers ($p < 0.02$) in the study population. Genotype definition as in Table 2. Data shown as mean value $\pm$ sd

is relied upon SHBG [6], also expressed in granulosa cells. Therefore, the reduced gene activity of the above factors would probably affect the follicular fluid protein content. In the present study, we sought to explore the potential synergistic effect of *FSHR* 307(T/A)/*FSHR* 680(N/S), *SHBG*(TAAAA)_n and *CYP19*(TTTA)_n polymorphisms on the ovarian response to standard gonadotrophin stimulation. Indeed, a gradual reduction in the follicle/oocyte number from *FSHR* 307Thr/680Asn allele/long *SHBG* allele/long *CYP19* allele homozygotes to *FSHR* 307Ala/680Ser allele/short *SHBG* allele/short *CYP19* allele homozygotes was observed, indicating the significance of these genes for the follicular growth and oocyte maturation during FSH stimulations.

The role of *FSHR* in the local regulation of ovarian function has been highlighted, studying female *FSHR*-knockout mice. These mice are characterized by smaller ovaries, atrophic uterus and imperforate vagina [20] probably due to the lack of circulating biologically active estrogen, while their elevated serum and pituitary FSH concentrations lead to follicular arrest at the primordial, primary and preantral stages [21, 22]. In the current study, the combined *FSHR*/*SHBG*/*CYP19* genotype analysis revealed increased follicle/oocytes numbers in Thr307Thr/Asn680Asn carriers compared to Ala307Ala/Ser680Ser carriers. These findings are in accordance with previous studies [12, 13, 23–28]. The higher follicle and oocyte numbers of Thr307Thr/Asn680Asn women compared to Ala307Ala/Ser680Ser women suggest a putative association of Ala307Ala/Ser680Ser genotype with a poorer response to controlled ovarian stimulation [12, 13, 23–25] and with a need of higher recombinant FSH units administration in Thr307Thr/Asn680Asn women [26–28]. Furthermore, Ser680Ser women have been characterized by longer follicular phase duration [27] compared to the Asn680Asn women, while the Ser680Ser genotype has been observed more frequently in women with ovarian dysfunction [25] and in normogonadotropic anovulatory infertile women [29]. Therefore, women with Ala307Ala/Ser680Ser diplotypes are possibly more resistant to FSH action and they may need increased amounts of gonadotrophins during COS so as to achieve follicle and oocytes numbers as high as those observed in Thr307Thr/Asn680Asn women.

On the other hand, the increased follicle/oocyte numbers of long *SHBG*(TAAAA)_n allele homozygotes compared to short *SHBG*(TAAAA)_n allele homozygotes, which were observed during the combined *FSHR*/*SHBG*/*CYP19* genotype analysis, put forward the potential significance of this polymorphism alone for the controlled ovarian stimulation outcome. Indeed, a previous study of our group has shown that women with short *SHBG*(TAAAA)_n genotypes have higher numbers of small follicles and decreased numbers of oocytes [11]. Clinical studies have shown that *SHBG*(TAAAA)_n polymorphism affects the transcriptional activity of the *SHBG* gene leading to variations of serum [30–32] and follicular [11] SHBG levels.

Specifically, healthy as well as polycystic ovary syndrome (PCOS) women with long *SHBG*(TAAAA)_n alleles have been characterized by lower SHBG levels. Low serum/follicular SHBG levels leave a considerable amount of androgens unbound and active, which promote both theca and granulosa cell proliferation [33, 34] leading to greater amounts of large follicles and oocytes. However, in the current study the combined *FSHR*/*SHBG*/*CYP19* genotypes did not show any association with serum SHBG levels at the third day of the menstrual cycle. Taking into consideration that the simultaneous analysis of the three polymorphisms causes a broad combined genotype distribution as well as the reduction in the number of the studied subjects due to the exclusion of heterozygotes, further studies in larger populations are needed to confirm the actual association of the combined *FSHR*/*SHBG*/*CYP19* genotypes with serum and/or follicular SHBG levels.

The critical role of *CYP19* in the local regulation of ovarian function has been found by studying female *CYP19*-knockout mice. These mice are characterized by hyperandrogenism, congenital genital ambiguity, hypergonadotropic hypogonadism, pubertal failure, virilisation, multicystic ovaries, breast development absence and infertility [35–38]. The combined *FSHR*/*SHBG*/*CYP19* genotype analysis of the current study showed increased follicle/oocyte numbers in long *CYP19*(TTTA)_n allele homozygotes compared to short *CYP19*(TTTA)_n allele homozygotes. Similar findings have been observed in two previous reports [12, 39]. Short *CYP19*(TTTA)_n allele carriers are probably in need of increased gonadotrophin administration during a controlled ovarian stimulation in order to achieve follicle numbers as high as those observed in long *CYP19*(TTTA)_n allele carriers. The increased estrogen biosynthesis in endometrial tumours of long *CYP19*(TTTA)_n allele homozygotes [40] as well as the higher testosterone/estradiol ratios of short *CYP19*(TTTA)_n allele PCOS homozygotes [10], have suggested a potential association of short *CYP19* alleles with reduced aromatase activity. A reduced aromatase activity would probably lead to androgen excess in utero, affecting negatively the anti-Müllerian hormone production by granulosa cells of growing pre-antral and small antral follicles and consequently the ovarian follicle cohort size and reserve [41, 42]. In the current study, although the combined *FSHR*/*SHBG*/*CYP19* genotypes were not associated with serum estradiol and testosterone levels, their implication in ovarian/follicular estrogen/androgen level regulation, needs to be clarified studying considerably larger population groups.

In the present study, the extremely decreased follicle/oocyte numbers of *FSHR*307Ala/680Ser allele/short *SHBG* allele/short *CYP19* allele homozygotes are probably found, due to the additive effect of the separate unfavorable genotypes. According to the literature, the Ala307Ala/Ser680Ser genotype has been associated with increased FSH levels, which in turn could affect negatively the synthesis of cytochrome P450 aromatase in granulosa cells [2]. Additionally,

the short *CYP19(TTTA)_n* alleles on their own, which have been associated with a reduced aromatase activity [10], could cause an extra impairment in the protein synthesis or sensitivity. Finally, the higher SHBG levels of short *SHBG(TAAAA)_n* homozygotes [11, 30] would probably reduce the levels of unbound/active androgens that could be used by cytochrome P450 aromatase for the biosynthesis of estrogens. Therefore, the consequent estrogen/androgen imbalance would probably lead to a defective maturation of follicles and to decreased follicle/oocyte numbers.

The above results put forward the association of *FSHR* 307 (T/A)/*FSHR* 680(N/S), *SHBG(TAAAA)_n* and *CYP19(TTTA)_n* combined genotypes with the ovarian response to standard gonadotrophin stimulation of women undergoing medically assisted reproduction and confirm the significance of *FSHR*, *SHBG* and *CYP19* genes for the female reproductive outcome under stimulation. After the validation of our results in larger patient groups, this combined genotype analysis could help in the selection of the proper stimulation protocol so as to achieve a sufficient number of mature oocytes.

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

References

- Gharib SD, Wierman ME, Shupnik MA, Chin WW. Molecular biology of pituitary gonadotropins. *Endocr Rev.* 1990;11:177–99.
- Simoni M, Nieschlag E. FSH in therapy: physiological basis, new preparations and clinical use. *Reprod Med Rev.* 1995;4:163–7.
- Simoni M, Gromoll J, Nieschlag E. The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology. *Endocr Rev.* 1997;18:739–73.
- Misao R, Nakanishi Y, Fujimoto J, Tamaya T. Expression of sex hormone-binding globulin mRNA in uterine leiomyoma, myometrium and endometrium of human subjects. *Gyn Endocrinol.* 1995;9:317–23.
- Forges T, Gerard A, Hess K, Monnier-Barbarino P, Gerard H. Expression of sex hormone-binding globulin (SHBG) in human granulosa-lutein cells. *Mol Cell Endocrinol.* 2004;219:61–8.
- Hammond GL, Bocchinfuso WP. Sex hormone-binding globulin/androgen-binding protein: steroid-binding and dimerization domains. *J Ster Biochem Mol Biol.* 1995;53:543–52.
- Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, et al. Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocr Rev.* 1994;15:342–55.
- Simoni M, Gromoll J, Hoppner W, Kamischke A, Kraft T, Stahle D, et al. Mutational analysis of the follicle-stimulating hormone receptor in normal and infertile men: identification and characterization of two discrete FSH receptor isoforms. *J Clin Endocrinol Metab.* 1999;84:751–5.
- Hogeveen KN, Talikka M, Hammond GL. Human sex hormone-binding globulin promoter activity is influenced by a (TAAAA)_n repeat element within an Alu sequence. *J Biol Chem.* 2001;276:36383–90.
- Xita N, Lazaros L, Georgiou I, Tsatsoulis A. CYP19 gene: a genetic modifier of polycystic ovary syndrome phenotype. *Fertil Steril.* 2010;94:250–4.
- Hatzi E, Bouba I, Galidi A, Lazaros L, Xita N, Sakaloglou P, et al. 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.
- Lazaros L, Hatzi E, Xita N, Makrydimas G, Kaponis A, Takenaka A, et al. Aromatase (CYP19) gene variants influence ovarian response to standard gonadotrophin stimulation. *J Assist Reprod Genet.* 2012;29:203–9.
- Lazaros L, Hatzi E, Xita N, Makrydimas G, Kaponis A, Plachouras N, Sofikitis N, Stefos T, Zikopoulos K, Georgiou I. Ovarian response to standard gonadotrophin stimulation for IVF/ICSI is influenced by *FSHR* diplotypes. 2012 *J Reprod Med* (in press).
- Droesch K, Muasher SJ, Brzyski RG, Jones GS, Simonetti S, Liu HC, et al. Value of suppression with a gonadotropin-releasing hormone agonist prior to gonadotropin stimulation for in vitro fertilization. *Fertil Steril.* 1989;51:292–7.
- Lazaros L, Xita N, Kaponis A, Zikopoulos K, Sofikitis N, Georgiou I. Evidence for association of Sex hormone-binding globulin and androgen receptor genes with semen quality. *Andrologia.* 2008;40:186–91.
- Xita N, Georgiou I, Lazaros L, Psafaki V, Kolios G, Tsatsoulis A. The synergistic effect of sex hormone-binding globulin and aromatase genes on polycystic ovary syndrome phenotype. *Eur J Endocrinol.* 2008;158:861–5.
- Baghaei F, Rosmond R, Westberg L, Hellstrand M, Eriksson E, Holm G, et al. The CYP19 gene and associations with androgens and abdominal obesity in premenopausal women. *Obes Res.* 2003;11:578–85.
- McNatty KP, Smith DM, Makris A, Osathanondh R, Ryan KJ. The microenvironment of the human antral follicle: interrelationships among the steroid levels in antral fluid, the population of granulosa cells, and the status of the oocyte in vivo and in vitro. *J Clin Endocrinol Metab.* 1979;49:851–60.
- Nayudu PL, Lapota A, Jones GM, Gook DA, Bourne HM, Sheather SJ, et al. An analysis of human oocytes and follicles from stimulated cycles: oocyte morphology and associated follicular fluid characteristics. *Hum Reprod.* 1989;4:558–67.
- Abel MH, Wootton AN, Wilkins V, Huhtaniemi I, Knight PG, Charlton HM. The effect of a null mutation in the FSH receptor gene on mouse reproduction. *Endocrinology.* 2000;141:1795–803.
- Abel MH, Huhtaniemi I, Pakarinen P, Kumar TR, Charlton HM. Age-related uterine and ovarian hypertrophy in FSH receptor knockout and FSH subunit knockout mice. *Reproduction.* 2003;125:165–73.
- Hirst RC, Abel MH, Wilkins V, Simpson C, Knight PG, Zhang F, et al. Influence of mutations affecting gonadotropin production or responsiveness on expression of inhibin subunit mRNA and protein in the mouse ovary. *Reproduction.* 2004;128:43–52.
- de Castro F, Ruiz R, Montoro L, Pérez-Hernández D, Sánchez-Casas Padilla E, Real LM, et al. Role of follicle-stimulating hormone receptor Ser680Asn polymorphism in the efficacy of follicle-stimulating hormone. *Fertil Steril.* 2003;80:571–6.
- Jun JK, Yoon JS, Ku SY, Choi YM, Hwang KR, Park SY, et al. Follicle-stimulating hormone receptor gene polymorphism and ovarian responses to controlled ovarian hyperstimulation for IVF-ET. *J Hum Genet.* 2006;51:665–70.
- Livshits G, Podlesnaja S, Kravchenko S, Sudoma I, Livshits L. A distribution of two SNPs in exon 10 of the *FSHR* gene among the women with a diminished ovarian reserve in Ukraine. *J Assist Reprod Genet.* 2009;26:29–34.
- Perez Mayorga M, Gromoll J, Behre HM, Gassner C, Nieschlag E, Simoni M. Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype. *J Clin Endocrinol Metab.* 2000;85:3365–9.

27. Théron-Gérard L, Pasquier M, Czernichow C, Cédric-Durnerin I, Hugues JN. Follicle-stimulating hormone receptor polymorphism and ovarian function. *Gynecol Obstet Fertil*. 2007;35:135–41.
28. Wunsch A, Ahda Y, Banaz-Yaşar F, Sonntag B, Nieschlag E, Simoni M, et al. Polymorphism of the FSH receptor and ovarian response to FSH. *Ann Endocrinol (Paris)*. 2007;68:160–6.
29. Laven JS, Mulders AG, Suryandari DA, Gromoll J, Nieschlag E, Fauser BC, et al. Follicle-stimulating hormone receptor polymorphisms in women with normogonadotropic anovulatory infertility. *Fertil Steril*. 2003;80:986–99.
30. Xita N, Tsatsoulis A, Chatzikiyiakidou A, Georgiou I. Association of the (TAAAA)_n repeat polymorphism in the sex hormone-binding globulin (SHBG) gene with polycystic ovary syndrome and relation to SHBG serum levels. *J Clin Endocrinol Metab*. 2003;88:5976–80.
31. Cousin P, Calemard-Michel L, Lejeune H, Raverot G, Yessaad N, Emptoz-Bonneton A, et al. Influence of SHBG gene pentanucleotide TAAAA repeat and D327N polymorphism on serum sex hormone-binding globulin concentration in hirsute women. *J Clin Endocrinol Metab*. 2004;89:917–24.
32. Ferik P, Teran N, Gersak K. The (TAAAA)_n microsatellite polymorphism in the SHBG gene influences serum SHBG levels in women with polycystic ovary syndrome. *Hum Reprod*. 2007;22:1031–6.
33. Takayama K, Fukaya T, Sasano H, Funayama Y, Suzuki T, Takaya R, et al. Immunohistochemical study of steroidogenesis and cell proliferation in polycystic ovarian syndrome. *Hum Reprod*. 1996;11:1387–92.
34. Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA. Androgens stimulate early stages of follicular growth in the primate ovary. *J Clin Invest*. 1998;101:2622–9.
35. Shozu M, Akasofu K, Harada T, Kubota Y. A new cause of female pseudohermaphroditism: placental aromatase deficiency. *J Clin Endocrinol Metab*. 1991;72:560–6.
36. 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 Metab*. 1994;78:1287–92.
37. 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 Metab*. 1995;80:3689–98.
38. Lin L, Ercan O, Raza J, Burren CP, Creighton SM, Auchus RJ, et al. Variable phenotypes associated with aromatase (CYP19) insufficiency in humans. *J Clin Endocrinol Metab*. 2007;92:982–90.
39. Altmäe S, Haller K, Peters M, Saare M, Hovatta O, Stavreus-Evers A, et al. Aromatase gene (CYP19A1) variants, female infertility and ovarian stimulation outcome: a preliminary report. *Reprod Biomed Online*. 2009;18:651–7.
40. Berstein LM, Imyanitov EN, Kovalevskij AJ, Maximov SJ, Vasilyev DA, Buslov KG, et al. CYP17 and CYP19 genetic polymorphisms in endometrial cancer: association with intratumoral aromatase activity. *Cancer Letters*. 2004;207:191–6.
41. Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome NP, Visser JA, et al. Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *Mol Hum Reprod*. 2004;10:77–83.
42. Seifer DB, Maclaughlin DT. Mullerian inhibiting substance is an ovarian growth factor of emerging clinical significance. *Fertil Steril*. 2007;88:539–46.