CLINICAL STUDY

The synergistic effect of sex hormone-binding globulin and aromatase genes on polycystic ovary syndrome phenotype

Nectaria Xita¹, Ioannis Georgiou², Leandros Lazaros², Vasiliki Psofaki³, George Kolios³ and Agathocles Tsatsoulis¹

¹Department of Endocrinology and ²Laboratory of Human Reproductive Genetics, University of Ioannina, Ioannina 45110, Greece and ³Laboratory of Biochemistry, University Hospital of Ioannina, Ioannina 45110, Greece

(Correspondence should be addressed to A Tsatsoulis; Email: atsatsou@uoi.gr)

Abstract

Objective: Experimental evidence suggests that fetal exposure to androgen excess may program the development of polycystic ovary syndrome (PCOS) in utero. The aim of this study was to examine whether the sex hormone binding globulin (SHBG)(TAAAA)*n* and the cytochrome P450, family 19 (CYP19)(TTTA)*n* polymorphisms, known to influence sex hormone-binding globulin (SHBG) levels and aromatase activity respectively, play a synergistic role in the development of PCOS.

Design and methods: We studied 180 women with PCOS and 160 healthy women of reproductive age. The body mass index (BMI) was recorded and the hormonal profile determined from the third to fifth day of menstrual cycle. DNA was extracted from blood leucocytes and the SHBG(TAAAA)*n* and CYP19(TTTA)*n* polymorphisms were genotyped.

Results: Genotype analysis revealed 6 SHBG(TAAAA)*n* alleles with 6–11 repeats and 6 CYP19(TTTA)*n* alleles with 7–12 repeats. Women were subdivided into four groups: those with short SHBG (≤ 8 TAAAA repeats) and CYP19 alleles (≤ 9 TTTA repeats), those with short SHBG–long CYP19 alleles, those with long SHBG–short CYP19 alleles, and those with long SHBG and CYP19 alleles. Women with PCOS tended to have at greater frequency, long SHBG–short CYP19 alleles compared with controls (57.3 vs 42.4%, $P=0.07$). Importantly, PCOS women with long SHBG–short CYP19 alleles had the lowest SHBG levels ($P=0.02$) and the highest total testosterone ($P=0.008$), free androgen index ($P=0.002$), DHEAS ($P=0.02$), and testosterone/estradiol ratio ($P=0.03$), compared with those with other genotypes. This association was independent of age, BMI, and insulin resistance indexes.

Conclusion: We speculate that the SHBG and CYP19 genes may have a synergistic role in the developmental programming of PCOS, by affecting androgen bioavailability and aromatization respectively.

European Journal of Endocrinology 158 861–865

Introduction

Hyperandrogenism is a key diagnostic feature of polycystic ovary syndrome (PCOS) and is a heritable PCOS trait. Sisters of women with PCOS have increased androgen levels suggesting that hyperandrogenism may be partly genetically determined (1). Furthermore, experimental animal research and clinical observations have led to the developmental origin hypothesis of PCOS, whereby exposure of the female fetus to androgen excess may program in utero the development of the PCOS phenotype in adult life (2, 3).

The potential origin of androgen excess during intrauterine life in humans, to account for fetal programming of PCOS, is not known. Normally, the female fetus is protected in utero from maternal androgens, or androgens from other source, by the increased placental sex hormone-binding globulin (SHBG) that binds the androgens and the aromatase activity that converts them to estrogens. However, this buffering effect may be overcome if either the production of SHBG or the aromatase activity

is lower than normal due to genetic or other influences. Based on these observations, polymorphisms of SHBG and aromatase cytochrome P450, family 19 (CYP19) genes may represent candidate genes contributing to the developmental origin of PCOS.

With regard to SHBG, we have previously shown a significant association between a functional (TAAAA)*n* repeat polymorphism of the SHBG gene with serum SHBG levels and PCOS phenotype, providing evidence for a possible genetic contribution for prenatal androgen excess (4). Similar findings were reported in other populations of women with hyperandrogenism (5, 6) and in a more recent study in young men (7).

On the other hand, regarding the CYP19 gene, women with congenital aromatase deficiency, caused by loss-of-function mutations of CYP19 gene, develop the features of PCOS phenotype (8, 9). Although earlier linkage and association studies failed to find an association between CYP19 and PCOS, probably due to low statistical power (10–12), a recent association study showed a strong association between a common

genetic variant (the single nucleotide polymorphism (SNP)50) in *CYP19* and androgen excess in girls with precocious puberty and functional hyperandrogenism (13). Furthermore, a recent study indicated that a short microsatellite (TTTA)*n* repeat allele in the fourth intron of the *CYP19* gene is associated with elevated androgens, perturbed regulation of the hypothalamic–pituitary–adrenal axis, and abdominal obesity among premenopausal women from the general population (14).

In the light of these observations, we hypothesized that the *SHBG* and the *CYP19* genes may interact in providing the androgenic stimulus for the developmental programming of PCOS. For this purpose, we examined the distribution of combined *SHBG*(TAAAA)*n* and *CYP19*(TTTA)*n* repeat variants between patients and controls and their association with the hyperandrogenic phenotype of PCOS.

Subjects and methods

Subjects

The study population consisted of 180 cases of PCOS and 160 age-matched healthy controls. The diagnosis of PCOS was based on the criteria proposed by the 1990 National Institutes of Health–National Institute of Child Health and Human Development conference on PCOS. These criteria are ovulatory dysfunction, clinical evidence of hyperandrogenism, and/or hyperandrogenemia and exclusion of related disorders such as congenital adrenal hyperplasia, hyperprolactinemia, or Cushing's syndrome (15). Hyperandrogenism was defined by the clinical presence of hirsutism (Ferriman–Gallwey score >8) acne or alopecia and/or elevated androgen levels. Menstrual dysfunction was defined by the presence of oligomenorrhea or amenorrhea. In those patients who were on medication, treatment was discontinued at least 6 months prior to their inclusion in the study. The control group consisted of women with normal menstrual cycles (28–30 days) and no signs of hyperandrogenism.

All patients were studied on days 3–5 of either a normal or progesterone-induced withdrawal period. The body mass index (BMI) of each patient, calculated as weight (kg)/height² (m), was recorded. Blood samples were drawn after overnight fasting for the measurement of fasting serum glucose and insulin, serum gonadotropins (luteinizing hormone, LH, follicle-stimulating hormone, FSH), total testosterone, estradiol, SHBG, and DHEAS. The free androgen index (FAI) was calculated using the formula: (total testosterone (nmol/l)/SHBG (nmol/l)) × 100 (16). Insulin resistance was assessed by the fasting glucose-to-insulin ratio (17). From both patients and controls, whole blood samples were used for isolation of peripheral blood leucocytes for the genetic analysis. The study protocol was approved by the Hospital Ethics Committee, and all subjects studied gave their informed consent.

Hormonal assays

Serum glucose was determined by the hexokinase method using a glucose analyzer (Olympus 600, Clinical Chemistry Analyser, Olympus Diagnostica GmbH, Co Clare, Ireland). The coefficient of variation (CV) of this method was <3%. Insulin was measured by microparticle enzyme immunoassay on an AXSYM Immunoanalyser (Abbott Laboratory). The CV of this method was 5%. Total testosterone and serum gonadotropins (LH, FSH) were determined by chemiluminescent microparticle immunoassay on an Abbott-ARCHITECT Immunoanalyser (Abbott Laboratory). The CV values were 4% for total testosterone, 3.5% for LH, and 4% for FSH. DHEAS and SHBG were measured by chemiluminescent immunometric method (IMMULITE 2000 Immunoanalyser, DPC, Los Angeles, CA, USA), and the CV values were 9 and 5.5% respectively.

Genotype analysis

Genomic DNA was isolated from peripheral blood leucocytes of women with PCOS and the controls. Amplification of the *SHBG*(TAAAA)*n* and *CYP19*(TTTA)*n* repeat region was accomplished using PCR with a forward primer (5'-GCTTGAACCTCGAGAGGCAG-3') and a reverse primer (5'-CAGGGCCTAAACAGTCTAG-CAGT-3') for the *SHBG*(TAAAA)*n* polymorphism and a forward primer (5'-GTTTGACTCCGTGAGTTTGA-3') and a reverse primer (5'-CAACTCGACCTTCTTTATG-3') for the *CYP19*(TTTA)*n* polymorphism. Amplified products were separated by 12% PAGE followed by silver staining and the number of repeats of each allele was determined. The number of TAAAA and TTTA repeats in every particular allele was analyzed by sequencing the appropriate PCR products. A quality control assessment of our PCR method was done by random sampling and sequencing of the PCR products and duplication of the PCR assays.

Statistical analysis

Statistical analysis of differences in genotype frequencies between PCOS and controls was performed using the χ^2 test. Normal distribution of continuous parameters was tested by Kolmogorov–Smirnov test. Differences in continuous parameters between genotypes were assessed with ANOVA test and confirmed with the non-parametric Kruskal–Wallis test. Stepwise multiple regression analysis was performed to identify independent predictors of androgen levels. $P < 0.05$ was considered statistically significant. All results are reported as mean $\pm$ s.d. All analyses used the SPSS statistical package (version 14.0, SPSS Inc., Chicago, IL, USA).

Results

Distribution of SHBG(TAAAA)*n* and CYP19(TTTA)*n* alleles

The characteristics of women with PCOS and the control group are presented in Table 1. As expected, PCOS women had higher mean BMI and insulin resistance indexes as well as lower SHBG and higher androgen levels compared with control women.

In both patients and controls, 6 SHBG(TAAAA)*n* alleles with 6–11 repeats and 6 CYP19(TTTA)*n* alleles with 7–12 repeats were identified. Comparing the frequencies of SHBG alleles between the two groups, long (TAAAA)*n* alleles (> 8 repeats) were more frequent in PCOS women than in controls, while shorter SHBG alleles (< 8 repeats) were more frequent among control women ($P=0.001$), as reported previously (4).

With regard to CYP19(TTTA)*n* alleles, women with PCOS tended to have more frequently short CYP19 alleles (≤ 9 repeats) than controls, although the difference did not reach statistical significance (33.1 vs 29.5%).

The study population was further subdivided into subgroups according to different combinations of the two gene polymorphic variants, using as cut-offs the (TAAAA)₈ allele of the SHBG gene and the (TTTA)₉ allele of the CYP19 gene respectively. According to this categorization, four subgroups were identified: women with short SHBG alleles (≤ 8 TAAAA repeats) and short CYP19 alleles (≤ 9 TTTA repeats), women with short SHBG–long CYP19 alleles, women with long SHBG–short CYP19 alleles, and women with long SHBG and CYP19 alleles. We found that in both PCOS women and controls, the combination of long SHBG–short CYP19 alleles was at high prevalence, compared with other genotype combinations. However, this combined genotype tended to be more frequent among PCOS women than healthy (57.3 vs 42.4%, $P=0.07$, Fig. 1). This difference in grouped genotype distribution was also observed when we compared lean PCOS women with lean controls (BMI < 25 kg/m², $P=0.06$) and overweight–obese PCOS women with overweight–obese

controls (BMI ≥ 25 kg/m², $P=0.09$). Subjects heterozygous for short and long alleles for both genes were not included in this analysis.

Association of combined SHBG–CYP19 polymorphisms with phenotypic variables in women with PCOS

Among the four genotype subgroups, patients with the combination of long SHBG–short CYP19 alleles (subgroup 3) had higher levels of total testosterone ($P=0.02$), FAI ($P=0.006$), DHEAS ($P=0.04$), and tended to have lower serum SHBG levels ($P=0.06$), compared with other genotype subgroups, while no difference was found in BMI and insulin resistance indexes between the different patient subgroups. Subjects heterozygous for short and long alleles for both genes were not included in this analysis.

When patients with long SHBG–short CYP19 alleles were compared with the total population of PCOS women (including heterozygotes for short and long alleles), it was found that this subgroup had the lowest serum SHBG levels ($P=0.02$) and the highest levels of total testosterone ($P=0.008$), FAI ($P=0.002$), DHEAS ($P=0.02$), and testosterone/estradiol ratio ($P=0.03$, Table 2). This association between genotype and androgen levels was independent of age, BMI, and insulin resistance indexes. Similarly, among the healthy women, the subgroup with long SHBG–short CYP19 alleles tended to have a higher androgen profile compared with other subgroups, although these differences were not statistically significant (results not shown).

Discussion

In the present study, we investigated whether the SHBG and CYP19 genes may exert a synergistic effect in the development of PCOS. In particular, we examined the combined effect of two functional polymorphisms, the SHBG(TAAAA)*n* and the CYP19(TTTA)*n* repeat polymorphisms, on the phenotypic expression of PCOS.

By subdividing the study population into subgroups according to the number of repeats of both SHBG and CYP19 polymorphisms, we found that the combination of long SHBG(TAAAA)*n*–short CYP19(TTTA)*n* alleles was at high prevalence in both PCOS women and controls, compared with other genotype combinations. However, this genotype combination was more frequent among PCOS women compared with control women, a difference that was close to being significant. More importantly, PCOS women who were carriers of the above genotype combination had a higher androgenic profile compared with patients with other genotype combinations.

Previous studies were consistent in showing that long SHBG(TAAAA)*n* alleles are associated with low SHBG and increased androgens in women with PCOS

Table 1 Anthropometric and biochemical characteristics of polycystic ovary syndrome (PCOS) women and controls.

	PCOS women	Controls	P value
Number	180	160	
Age (years)	23.7 $\pm$ 6.4	25.1 $\pm$ 6.0	NS
BMI (kg/m ²)	26.6 $\pm$ 6.9	23.0 $\pm$ 3.1	<0.05
LH/FSH	1.4 $\pm$ 1.0	1.1 $\pm$ 0.8	NS
SHBG (nmol/l)	37.9 $\pm$ 26.4	60.5 $\pm$ 26.7	<0.001
FAI	13.7 $\pm$ 10.4	3.4 $\pm$ 1.4	<0.001
Total testosterone (ng/ml)	0.98 $\pm$ 0.44	0.4 $\pm$ 0.1	<0.001
DHEAS (ng/ml)	2838 $\pm$ 1155	2001 $\pm$ 900	<0.001
Fasting glucose/insulin	10.3 $\pm$ 7.4	14.4 $\pm$ 5.2	<0.001

NS, non significant; values presented as means $\pm$ s.d.

Figure 1 Distribution of grouped *SHBG*(TAAAA)*n* and *CYP19*(TTTA)*n* alleles in PCOS women compared with controls. Short *SHBG* alleles: ≤ 8 TAAAA repeats, long *SHBG* alleles: > 8 TAAAA repeats, short *CYP19* alleles: ≤ 9 TTTA repeats, long *CYP19* alleles: > 9 TTTA repeats. Heterozygotes for short and long alleles were not included in the analysis.

or hyperandrogenic women (4–6). On the other hand, the *CYP19*(TTTA)*n* polymorphism has been shown to influence aromatase activity. In particular, lower aromatase activity was reported in fibroblasts obtained from subjects homozygous for the low (TTTA)*n* genotype of *CYP19* than in those from subjects homozygous for a high TTTA repeat genotype (18, 19). In this context, a recent study reported an association between short *CYP19*(TTTA)*n* repeat alleles with increased androgens and abdominal obesity among premenopausal women in the general population (14).

The present study is the first to show an interactive effect of *SHBG* and *CYP19* gene polymorphisms on the phenotype of PCOS. In particular, the co-existence of long *SHBG*(TAAAA)*n*–short *CYP19*(TTTA)*n* alleles appears to contribute in a synergistic manner to the hyperandrogenism of PCOS, which is the fundamental manifestation of the syndrome.

The findings of this study have two potential implications. First, regarding the *CYP19* gene, the findings revive an old hypothesis that PCOS results from partial aromatase deficiency (20). Based on the earlier reports for an increased ratio of androstenedione to estradiol in the follicular fluid (21), Gabrilove

suggested, in a classification of virilizing and feminizing syndromes, that PCOS be categorized as resulting from decreased conversion of androgens to estrogens (22). This concept was also hinted at later on by Grumbach & Auchus (23) and Bulun (24) but was dismissed by others (25), mainly because of failure to find linkage of PCOS with the *CYP19* gene (11). The present study offers a possible explanation of this hypothesis.

Secondly, perhaps the more important but related implication is that the combined long *SHBG*–short *CYP19* genotype may contribute to the developmental programming of PCOS. Since increased *SHBG* and aromatase activity constitute the main fetoplacental barrier protecting the female fetus from maternal androgens, the presence of this particular genotype may confer a less efficient barrier function, allowing the exposure of the developing fetus to androgen excess. This in turn may exert a 'programming' effect for the development of PCOS phenotype in adult life. Interestingly, in this regard, the ovaries of aromatase knockout mice exhibit an increased interstitium with the presence of theca cells that morphologically resemble Leydig cells (26).

Supporting evidence for this hypothesis comes from the findings in our study that PCOS women with the

Table 2 Differences in biochemical parameters between patients with long sex hormone-binding globulin (*SHBG*) alleles (> 8 TAAAA repeats) and short *CYP19* alleles (≤ 9 TTTA repeats) and patients with other genotype subgroups.

	Patients with long <i>SHBG</i> and short <i>CYP19</i> alleles	Other patient subgroups	P value
Number	43	137	
Age (years)	21.0 $\pm$ 5.2	22.4 $\pm$ 6.0	NS
BMI (kg/m ²)	27.6 $\pm$ 5.8	28.1 $\pm$ 7.9	NS
LH/FSH	1.3 $\pm$ 0.8	1.4 $\pm$ 1.1	NS
SHBG (nmol/l)	31.5 $\pm$ 20.4	39.5 $\pm$ 27.6	0.02
FAI	16.5 $\pm$ 10.5	12.5 $\pm$ 12.1	0.002
Total testosterone (ng/ml)	1.1 $\pm$ 0.4	0.9 $\pm$ 0.4	0.008
DHEAS (ng/ml)	3292.8 $\pm$ 1279.2	2691.3 $\pm$ 1077.5	0.02
Total testosterone (ng/ml)/E ₂	0.024 $\pm$ 0.002	0.016 $\pm$ 0.015	0.03
Fasting glucose/insulin	10.1 $\pm$ 10	10.4 $\pm$ 6.0	NS

NS, non statistical significant; values presented as means $\pm$ s.d.

combined long *SHBG*–short *CYP19* genotype exhibit not only increased FAI and testosterone/estradiol ratio, as would be expected from the low SHBG levels and reduced aromatase activity respectively, but also increased total testosterone and DHEAS levels. These findings indicate the presence of functional ovarian and adrenal hyperandrogenism that was probably programmed in utero by androgen excess.

In conclusion, the findings of the present study support the hypothesis that *SHBG* and *CYP19* gene variants play a synergistic role in the hyperandrogenic phenotype of PCOS, by affecting androgen bioavailability and aromatization respectively. Whether this combined genotype may contribute to the developmental programming of PCOS is a hypothesis requiring further substantiation.

Acknowledgements

Part of this article was presented to the annual meeting of the Hellenic Endocrine society in 2007 and received the Hellenic Endocrine Society Award.

References

- Legro RS, Driscoll D, Fox J III, Strauss JF & Dunaif A. Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. *PNAS* 1998 **95** 14956–14960.
- Abbott DH, Dumesic DA & Franks S. Developmental origin of polycystic ovary syndrome – a hypothesis. *Journal of Endocrinology* 2002 **174** 1–5.
- Xita N & Tsatsoulis A. Fetal programming of polycystic ovary syndrome by androgen excess: evidence from experimental, clinical, and genetic association studies. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 1660–1666.
- Xita N, Tsatsoulis A, Chatzikyriakidou 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 levels. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 5976–5980.
- Cousin P, Calemard-Michel L, Lejeune H, Raverot G, Yessaad N, Emptoz-Bonneton A, Morel Y & Pugeat M. Influence of SHBG gene pentanucleotide TAAAA repeat and D327N polymorphism on serum sex hormone-binding globulin concentration in hirsute women. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 917–924.
- Ferk P, Teran N & Gersak K. The (TAAAA)*n* microsatellite polymorphism in the SHBG gene influences serum SHBG levels in women with polycystic ovary syndrome. *Human Reproduction* 2007 **22** 1031–1036.
- Turk A, Kopp P, Colangelo LA, Urbanek M, Wood K, Liu K, Skinner HG & Gapstur SM. Associations of serum sex hormone-binding globulin (SHBG) levels with SHBG gene polymorphisms in the CARDIA Male Hormone Study. *American Journal Epidemiology* 2007 **167** 412–418.
- Ito Y, Fisher CR, Conte FA, Grumbach MM & Simpson ER. Molecular basis of aromatase deficiency in an adult female with sexual infantilism and polycystic ovaries. *PNAS* 1993 **90** 11673–11677.
- 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. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 5127–5131.
- Gharani N, Waterworth DM, Batty S, White D, Gilling-Smith C, Conway GS, McCarthy M, Franks S & Williamson R. Association of the steroid synthesis gene *CYP11a* with polycystic ovary syndrome and hyperandrogenism. *Human Molecular Genetics* 1997 **6** 397–402.
- Urbanek M, Legro RS, Driscoll DA, Azziz R, Ehrmann DA, Norman RJ, Strauss JF III, Spielman RS & Dunaif A. Thirty-seven candidate genes for polycystic ovary syndrome: strongest evidence for linkage is with follistatin. *PNAS* 1999 **96** 8573–8578.
- Soderlund D, Canto P, Carranza-Lira S & Mendez JP. No evidence of mutations in the P450 aromatase gene in patients with polycystic ovary syndrome. *Human Reproduction* 2005 **20** 965–969.
- Petry CJ, Ong KK, Michelson KF, Artigas S, Wingate DL, Balen AH, de Zegher F, Ibanez L & Dunger DB. Association of aromatase (*CYP19*) gene variation with features of hyperandrogenism in two populations of young women. *Human Reproduction* 2005 **20** 1837–1843.
- Baghaei F, Rosmond R, Westberg L, Hellstrand M, Eriksson E, Holm G & Bjorntorp P. The *CYP19* gene and associations with androgens and abdominal obesity in premenopausal women. *Obesity Research* 2003 **11** 578–585.
- Zawadzki JK & Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In *Polycystic Ovary Syndrome*, pp 377–384. Eds A Dunaif, JR Givens, F Haseltine & GR Merriam, Boston: Blackwell, 1992.
- Clark AF, Marcellus S, deLory B & Bird CE. Plasma testosterone free index: a better indicator of plasma androgen activity? *Fertility and Sterility* 1975 **26** 1001–1005.
- Legro RS, Finegood D & Dunaif A. A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 2694–2698.
- Kristensen VN, Harada N, Yoshimura N, Haraldsen E, Lønning PE, Erikstein B, Karesen R, Kristensen T & Borresen-Dale AL. Genetic variants of *CYP19* (aromatase) and breast cancer risk. *Oncogene* 2000 **19** 1329–1333.
- Gennari L, Masi L, Merlotti D, Picariello L, Falchetti A, Tanini A, Mavilia C, Del Monte F, Gonnelli G, Lucani B, Gennari C & Brandi ML. A polymorphic *CYP19* TTTA repeat influences aromatase activity and estrogen levels in elderly men: effects on bone metabolism. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 2803–2810.
- Gabrilove JL. The pathogenesis of the polycystic ovary syndrome: a hypothesis. *Endocrine Practice* 2002 **8** 127–132.
- Short RV & London DR. Defective biosynthesis of ovarian steroids in the Stein-Leventhal syndrome. *BMJ* 1961 **1** 1724–1727.
- Gabrilove JL. Diseases associated with some enzymic defects in the gonads and adrenal cortex; a classification based on a theory of the biogenesis of the feminizing and virilizing syndromes. *Journal of the Mount Sinai Hospital, New York* 1964 **31** 449–456.
- Grumbach MM & Auchus RJ. Estrogen: consequences and implications of human mutations in synthesis and action. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 4677–4694.
- Bulun SE. Clinical Review 78: aromatase deficiency in women and men; would you have predicted the phenotypes? *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 867–871.
- Ehrmann DA, Barnes RB & Rosenfield RL. Polycystic ovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. *Endocrine Reviews* 1995 **16** 322–353.
- Britt KL & Findlay JK. Estrogen actions in the ovary revisited. *Journal of Endocrinology* 2002 **175** 269–276.

Received 19 February 2008

Accepted 5 March 2008