Impact of estrogen receptor α gene and oxytocin receptor gene polymorphisms on female sexuality

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Abstract

Over the past decades, research attention has increasingly been paid to the neurobiological component of sexual behavior. The aim of the present study was to investigate the correlation of estrogen receptor α (*ERA*) gene polymorphism (rs2234693-PvuII) (T→C substitution) and oxytocin receptor gene polymorphism (rs53576) (G→A substitution) with sexuality parameters of young, healthy women. One hundred thirty-three Greek heterosexual women, students in higher education institutions, 20–25 years of age, sexually active, with normal menstrual cycles (28–35 days), were recruited in the study. Exclusion criteria were chronic and/or major psychiatric diseases, use of oral contraceptive pills (OCs), polycystic ovary syndrome (PCOS), thyroid diseases as well as drugs that are implicated in hypothalamus–pituitary–gonadal axis. T allele (wildtype) of rs2234693 (PvuII) polymorphism of *ERA* gene was correlated with increased levels of arousal and lubrication, whereas A allele (polymorphic) of rs53576 (*OXTR*) polymorphism was correlated with increased arousal levels. The simultaneous presence of both T allele of rs2234693 (PvuII) and A allele of rs53576 (*OXTR*) polymorphisms (T + A group) was correlated with increased arousal, orgasm levels as well as female sexual function index full score. To our knowledge, this is the first study to investigate the interaction between *ERA* and *OXTR* with regard to sexual function in women. Female sexuality is a complex behavioral trait that encompasses both biological and psychological components. It seems that variability in female sexual response stems from genetic variability that characterizes endocrine, neurotransmitter and central nervous system influences.

Key Words
- estrogen receptor α gene polymorphism
- oxytocin receptor gene polymorphism
- female sexuality

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Introduction

Over the past decades, research attention has been increasingly paid to the examination of the neurobiological component of sexual function.

Female sexual desire and arousal have been shown to have a heritable component of moderate size. Data have shown that additive and non-additive genetic effects explained 21–35% of the variation in desire, 24–26% in subjective arousal and 16–25% in lubrication (1, 2).

Central regulatory role of estrogens in female sexuality is unequivocal. Estrogens exert a direct effect on hypothalamic neurons at hyperchiasmatic level, especially on paraventricular nucleus (PVN), ventromedial nucleus (VMN), cortical areas (occipital–temporal

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cortex, ventral procinetic cortex and medial prefrontal cortex), hippocampus, central nucleus of amygdala and pedunculopontine nucleus (PPN) (3, 4, 5, 6, 7). Furthermore, peripheral actions of estrogens also promote female sexuality, including vaginal trophic effects as well as increase in skin sensitivity (8).

Estrogens binding to estrogen receptors (ERA or ERB) activate estrogen-responsive genes and stimulate ER-positive cell lines. Although single-nucleotide polymorphisms (SNPs) of *ERB* gene (rs1271572, rs4986938 and rs928554) have been associated with sexual desire and lubrication (9), the involvement of *ERA* gene polymorphisms in the variation of female sexual functioning has not been evidenced (9, 10). Rs2234693 (PvuII) polymorphism is a common SNP (42% in Caucasians), located in intron 1 of *ERA* gene in which thymine is substituted by cytosine (T→C). The presence of the dominant T allele of PvuII polymorphism can enhance ERA activity (11). Although increased reproductive efficiency has been associated with wildtype allele of PvuII polymorphic site (12), the implication of rs2234693 has not been associated with sexual function.

Oxytocin is a peptide associated with behavioral and psychological traits such as enhancement of pair bonding and affiliation; establishment of social bonds; decrease of social anxiety and increase of empathy, emotion recognition and interpersonal trust (13). Oxytocin is detected in peripheral tissues such as uterus, placenta, corpus luteum, playing a role in lactation and in contraction of the smooth muscle of the uterus during parturition. Furthermore, oxytocin is detected in the central nervous system (CNS), mainly in hypothalamic areas (paraventricular PVN and supraoptic SON nuclei, bed nucleus of the stria terminalis-BNST and in medial prooptic area-MPOA) and several nuclei of the amygdala.

Oxytocin is implicated both in social behavioral network (14, 15) and in sexual response. Increased oxytocin levels have been reported during sexual arousal, reaching peak levels during orgasm, whereas increased oxytocin levels have been confirmed immediately after orgasm. Furthermore, oxytocin levels have positively been correlated with intensity of contractions of the pelvic musculature during orgasm (16, 17, 18, 19).

Oxytocin receptor (*OXTR*) is a 389 amino acid polypeptide with 7 transmembrane domains that belong to the class I G protein-coupled receptor (GPCR) family (20). Rs53576 is an SNP substitution of guanine by adenine (G→A), which has thoroughly been studied and linked to socially related personality traits and behaviors (21).

Sexual response is attributed to coordinated genital reflexes that are influenced by estrogens and oxytocin. The lumbosacral spinal cord is the final source of output to the genital musculature, whose activity is modulated by a number of descending brain systems that regulate somatic genital reflexes. Certain brain areas of the thalamus seem to play a stimulatory role in the coordination of rhythmic contractions of the genital musculature via these lumbosacral targets. These thalamic areas are the spinothalamic neural cells in the lumbar spinal cord (LSt), in conjunction with the subparafascicular parvocellular nucleus (SPFpc) of the thalamus. On the other hand, the lumbosacral targets are under tonic inhibition provided by the nucleus paragigantocellularis (nPGi), which receives projections from hypothalamic nuclei (MPOA and PVN) and the periaqueductal gray matter (PAG). Estrogen receptors have been detected in MPOA, PVN and PAG nuclei, illustrating the strong estrogenic input of these areas. Ascending genitosensory information has been shown to influence these lumbosacral targets either directly or indirectly via brain regions associated with the regulation of sexual behavior, including the oxytocin receptors-containing neurons in the PVN (22).

Although female sexuality has been associated with *ERB* gene polymorphisms, *ERA* gene polymorphisms have not been related to sexuality parameters (9, 10). In fact, the estrogen effect on sexuality is the result of ERA and ERB interaction. Consequently, the role of ERA in female sexuality is crucial compared to that of ERB, given that *ERA* knockout mice did not display sexual behavior, whereas *ERB* knockout mice exhibited lordosis behavior equal to wildtype ones (23).

The importance of female sexuality in investigating genetic components lies in the organizational effects of estradiol and oxytocin on human CNS. Behaviors that have traditionally been associated with oxytocin's acute neuromodulatory effects, such as affiliative and sexual behaviors have been shown to establish a long-lasting pattern depending on the exposure pattern of oxytocin in early postnatal development and during puberty (14). Furthermore, it seems that estradiol has organizational effects on certain behaviors. The old concept that female differentiation develops in the absence of any hormonal influence has been re-examined, as it has been shown that estradiol is required during development for the expression of sociosexual behaviors in adult female mice (24).

We could hypothesize that *ERA* gene polymorphism rs2234693 and *OXTR* gene polymorphism rs53576 could be implicated on human sexuality parameters.

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Our hypothesis was based on previous findings relating rs2234693 to increased affinity for estrogens as well as to higher circulating estrogen levels (25). In addition, research into humans has underlined the strong involvement of rs53576 on social functions such as parenting, empathy and using of social relationships to manage stress (26). Indeed, a regulatory interplay between oxytocinergic system and gonadal steroids is well established. Estrogens have been shown to increase oxytocin receptor binding (27), whereas oxytocin has been shown to increase expression of ERα (28). Therefore, the interaction of these two genetic polymorphisms could have an additive effect on female sexuality.

The aim of the present study was to investigate the correlation of estrogen receptor α (*ER*α) gene polymorphism (rs2234693-PvuII) and oxytocin receptor gene polymorphism (rs53576) with sexuality parameters of young healthy women. This is the first study to investigate the interaction between *ERA* and *OXTR* in relation to sexual function in women.

Subjects and methods

Initially, one hundred ninety-four Greek heterosexual women aged 20–25 years old, sexually active, voluntarily participated in the study. One hundred women were students of childbirth assistance of Technological Educational Institute of Athens, whereas ninety-four women were medical students of University of Patras Medical School. Women were recruited after their attendance of two workshops on female sexuality organized by the Division of Reproductive Endocrinology, Department of Obstetrics and Gynaecology of the Medical School, University of Patras. Exclusion criteria were chronic diseases, major psychiatric disorders, use of oral contraceptive pills (OCs), polycystic ovary syndrome (PCOS), thyroid diseases as well as drugs that are implicated in hypothalamus–pituitary–gonadal axis. Women on OCs or on drugs that are implicated in hypothalamus–pituitary–gonadal axis were excluded due to their inhibited ovarian hormone secretion. Psychiatric diseases are a possible co-variant of sexuality parameters, and thus, they should be taken into account in a model constructed by multiple regression. In our study, only genetic components were examined and the study population should be homogeneous, clearly having or not having psychiatric morbidity. Consequently, women with major psychiatric disorders were excluded from the study. Due to disclosed hyperandrogenism and/or anovulation in 61 women, only 133 women with normal ovulatory menstrual cycles (28–35 days) were finally enrolled in the study. All eligible participants were students in higher education institutions and gave written informed consent before study entry. The conduct of the study was approved by the Institutional Review Board of Patras Medical School, functioning according to the 3rd edition of the Guidelines on the Practice of Ethical Committees in Medical Research issued by the Royal College of Physicians of London.

Height and weight were measured, and body mass index (BMI) (kg/m^2) was calculated. Hormonal determinations of follicular (1st–4th day of menstrual cycle) and luteal phase (18th–21st day) were conducted. Luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, testosterone and sex hormone-binding globulin (SHBG) were measured, whereas Free Androgen Index (FAI) was calculated on follicular phase. Progesterone levels were measured on luteal phase of menstrual cycle. LH, FSH and testosterone were determined based on Chemiluminescence (Elecsys 2010, Roche Diagnostics), whereas SHBG and progesterone were measured by RIA (BioSource, B-1400 Nivelles-Belgium).

Questionnaires

Sexual function was measured by the Greek version of the Female Sexual Function Index (FSFI) (29), a 19-item multiple choice questionnaire, which we have used in previous research (30, 31, 32). The FSFI is an internationally accepted and reliable instrument for rating female sexual function for research or clinical use (33). It measures six domains, including sexual desire, arousal (subjective), lubrication, orgasm, satisfaction and pain over the past 4 weeks. The six domain scores are summed to produce a full-scale score. For all FSFI domains, higher values indicate a better level of function (29). Moreover, age of adrenarche, menarche, age of first intercourse and also number of sexual partners were included in the questionnaires.

Study of genetic polymorphisms

DNA extraction was conducted by leukocytes of peripheral blood using the method of phenol. Study of rs2234693(T→C) polymorphism of estrogen receptor α (*ER*α) gene was realized by amplification of genetic sequence using the method of polymerase chain reaction (PCR). The primers were: (ER-F): 5′-CTG CCA CCC TAT

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CTG TAT CTT TTC CTA TTC TCC-3′ and (ER-R): 5′-TCT TTC TCT GCC ACC CTG CGT CGA CCA TCT GA-3′. Reactions were carried out using the following parameters (35 cycles): 94°C for 4 min, 94°C for 45 s, 58°C for 45 s and 72°C for 1 min. Amplification was confirmed by gel agarose electrophoresis. Detection of rs2234693 polymorphism was conducted using restriction enzyme PvuII following gel agarose electrophoresis.

Study of rs53576(G→A) polymorphism of oxytocin receptor gene (*OXTR*) was conducted using PCR method. The primers were F: 5′-GCC CAC CAT GCT CTC CAC ATC-3′ και R: 5′-GCT GGA CTC AGG AGG AAT AGG GAC-3′. Amplification was confirmed by gel agarose electrophoresis. Detection of rs53576 polymorphism was conducted using restriction enzyme BamHI.

Statistical analysis

Statistical analysis was conducted using SPSS 15.0 for Windows (IBM SPSS Statistics, IBM software). Test of normality for distribution of variables was done by Kolmogorov–Smirnov test. All parameters are presented as mean value (±S.D. value) (mean ± S.D.), regardless of their distribution. Study of Hardy–Weinberg equilibrium between observed and expected genotype frequencies was done using χ^2 goodness-of-fit test. The sample was in Hardy–Weinberg equilibrium for a polymorphism when $P > 0.05$. The comparison of mean values among three genotype groups was analyzed with one-way ANOVA for normally distributed variables and the Kruskal–Wallis one-way ANOVA for non-normally distributed variables. The comparison of mean values between two genotype groups was done by independent samples *t*-test for normally distributed variables and the Mann–Whitney test for non-normally distributed variables. Two-tailed significance level was set at 5%. The effect sizes of normal distributions were calculated, based on the equation $r = V(t^2/(t^2 + df))$, where *t*-statistic was preferred for both *t*-tests and contrasts of ANOVA, comparing only two things per test, and *df* was degrees of freedom. Similarly, *z*-score was used for calculating

Table 1 Anthropometric and hormonal characteristics ($N = 133$).

Variable	Mean (S.D.)
Age	21.62 (1.92)
Height (m)	1.64 (0.05)
Weight (kg)	59.88 (12.14)
Body mass index (BMI)	22.09 (4.00)
Estradiol (E_2) (pg/mL)	43.64 (15.20)
Testosterone (ng/mL)	0.31 (0.08)
FAI (%)	1.98 (1.40)
LH (IU/mL)	5.94 (2.20)
FSH (IU/mL)	6.53 (1.54)
SHBG (nmol/L)	69.54 (29.78)
Progesterone (ng/mL)	9.76 (7.39)

Table 2 Hormonal characteristics and sexual parameters among rs2234693 (PvuII) genotype groups (homozygous for T wild-type allele, homozygous for C polymorphic allele and heterozygous) and rs53576 (*OXTR*) genotype groups (homozygous for G wild-type allele and carriers of A polymorphic allele) (bold indicates significance level: $P < 0.05$).

	ERA				*OXTR*		
	TT ($N = 38$)	TC ($N = 64$)	CC ($N = 31$)	*P* value	GG ($N = 64$)	GA/AA ($N = 69$)	*P* value
Age	21.42 (2.04)	21.62 (1.96)	21.87 (1.72)	0.299	21.68 (2.08)	21.56 (1.78)	0.987
FSH (IU/mL)	6.57 (1.32)	6.48 (1.77)	6.57 (1.32)	0.952	6.64 (1.58)	6.42 (1.51)	0.402
LH (IU/mL)	5.98 (2.46)	5.89 (2.18)	5.98 (1.97)	0.84	5.91 (2.14)	5.97 (2.28)	0.861
E2 (pg/mL)	43.46 (14.30)	43.50 (14.64)	44.14 (17.71)	0.999	44.46 (16.52)	42.87 (13.94)	0.804
Progesterone (ng/mL)	9.29 (8.35)	10.03 (6.75)	9.79 (7.62)	0.496	9.88 (7.79)	9.66 (7.05)	0.941
Testosterone (ng/dL)	0.32 (0.08)	0.31 (0.08)	0.30 (0.09)	0.61	0.32 (0.08)	0.30 (0.09)	0.183
SHBG (nmol/L)	64.51 (30.91)	70.72 (31.49)	73.27 (24.32)	0.237	68.07 (29.9)	70.90 (29.82)	0.415
FAI	2.24 (1.54)	1.98 (1.49)	1.68 (0.95)	0.126	2.07 (1.44)	1.90 (1.37)	0.208
Desire	4.46 (0.86)	4.19 (0.92)	4.10 (1.07)	0.252	4.11 (0.96)	4.37 (0.92)	0.155
Arousal	5.48 (0.48)	4.96 (1.31)	4.61 (1.38)	**0.004**	4.95 (1.13)	5.10 (1.24)	**0.04**
Lubrication	5.58 (0.66)	5.22 (1.33)	5.03 (1.25)	**0.046**	5.27 (1.19)	5.29 (1.15)	0.993
Orgasm	5.15 (0.96)	4.66 (1.53)	4.55 (1.50)	0.211	4.66 (1.40)	4.88 (1.39)	0.152
Satisfaction	5.44 (0.90)	5.20 (1.20)	4.96 (1.06)	0.058	5.25 (1.14)	5.17 (1.06)	0.39
Pain	4.88 (1.46)	5.02 (1.61)	4.82 (1.53)	0.521	5.05 (1.42)	4.83 (1.65)	0.543
FSFI_full	31.02 (3.21)	29.27 (6.64)	28.10 (6.26)	0.075	29.32 (5.71)	29.67 (5.97)	0.265
Menarche	12.36 (1.14)	12.30 (1.35)	12.45 (1.31)	0.482	12.46 (1.43)	12.26 (1.12)	0.632
Adrenarche	10.36 (1.23)	10.34 (1.35)	10.25 (1.54)	0.769	10.56 (1.51)	10.11 (1.18)	0.172
Age of first intercourse	17.84 (1.91)	17.89 (1.99)	17.70 (1.73)	0.915	18.06 (1.91)	17.62 (1.87)	0.095
Number of sex partners	3.84 (4.73)	3.67 (3.58)	2.93 (2.36)	0.785	3.46 (4.26)	3.62 (3.16)	0.442

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effect sizes of non-normal distributions, based on the equation $r=z/\sqrt{N}$, where N represented the total number of observations. The statistics evaluating the associations between the individual SNPs and the outcome are of primary interest, and the type I error inflation due to multiple comparisons is typically addressed by procedures that control the family-wise error rate (such as the Bonferroni correction) or by determining the false discovery rates and q values. Consequently, the threshold for overall significance will be lower, thereby increasing power. It has been argued that correction for multiple testing in hypothesis-driven candidate gene association studies are too strict (34), and it was not conducted in the present study.

Results

Anthropometric and hormonal values of the sample are presented in Table 1. Mean values of testosterone and estradiol were within normal range, whereas women were normovulatory. Allele frequencies for each one of the polymorphisms were the following: concerning rs2234693 (*ERA*-PvuII), frequency of T allele (wildtype) was 52.6%, whereas frequency of C allele was 47.4%. Concerning rs53576 (*OXTR*), frequency of G allele (wildtype) was 71.8%, whereas frequency of A allele was 28.2% (data not shown). It was shown that rs2234693 (*ERA*-PvuII) and rs53576 (*OXTR*) polymorphisms were in Hardy–Weinberg equilibrium.

It was also shown that T allele (wildtype) of rs2234693 (PvuII) polymorphism was correlated with increased arousal levels ($P=0.004$, $r=0.39$, medium effect) and increased lubrication levels ($P=0.046$, $r=0.31$, medium effect) (Table 2).

Moreover, it was shown that A allele (polymorphic) of rs53576 (*OXTR*) polymorphism was correlated with increased arousal levels ($P=0.040$, $r=0.17$, small effect) (Table 2).

The simultaneous presence of both T allele of rs2234693 (PvuII) and A allele of rs53576 (*OXTR*) polymorphisms (T+A group) was correlated with increased arousal levels ($P=0.004$, $r=0.26$, medium effect), increased orgasm levels ($P=0.033$, $r=0.18$, small effect) and increased FSFI_full levels ($P=0.026$, $r=0.19$, small effect). There was a statistical trend for increased desire levels in the T+A group ($P=0.075$) (Table 3).

The presence of T allele of rs2234693 (PvuII) polymorphism concurrently with the absence of A allele of rs53576 (*OXTR*) polymorphism was correlated with increased satisfaction levels ($P=0.039$, $r=0.26$, medium effect), increased FAI levels and decreased SHBG levels compared to carriers of A allele of rs53576 (*OXTR*) polymorphism concomitantly with the absence of T allele of rs2234693 (PvuII) polymorphism (Table 3).

Table 3 Comparison of hormonal characteristics and sexual parameters between women who carry both T allele of rs223493 (PvuII) and A allele of rs53576 (*OXTR*) (T+A group) and the no (T+A) group and between women who carry T allele and no A allele (T+no A group) and those who carry A allele and no T allele (no T+A group) (bold indicates significance level: $P<0.05$).

	T+A ($N=53$)	**No (T+A)** ($N=80$)	***P* value**	**T+no A** ($N=49$)	**No T+A** ($N=16$)	***P* value**
Age	21.49 (1.89)	21.71 (1.95)	0.469	21.61 (2.09)	21.81 (1.37)	0.264
FSH (IU/mL)	6.37 (1.60)	6.63 (1.50)	0.351	6.67 (1.62)	6.57 (1.20)	0.827
LH (IU/mL)	5.95 (2.42)	5.93 (2.06)	0.515	5.90 (2.14)	6.02 (1.80)	0.594
E2 (pg/mL)	43.12 (13.20)	43.99 (16.46)	0.748	43.88 (15.81)	42.07 (16.59)	0.695
Progesterone (ng/mL)	9.66 (6.98)	9.83 (7.69)	0.856	9.86 (7.81)	9.65 (7.50)	0.897
Testosterone (ng/dL)	0.30 (0.09)	0.31 (0.08)	0.691	0.32 (0.07)	0.28 (0.09)	0.101
SHBG (nmol/L)	67.45 (29.85)	70.92 (29.84)	0.499	69.44 (33.01)	82.33 (27.61)	**0.049**
FAI	2.05 (1.47)	1.94 (1.36)	0.748	2.10 (1.56)	1.40 (0.86)	**0.019**
Desire	4.44 (0.88)	4.11 (0.97)	**0.075**	4.12 (0.91)	4.12 (1.02)	0.92
Arousal	5.28 (1.02)	4.86 (1.26)	**0.004**	5.02 (1.18)	4.51 (1.69)	0.464
Lubrication	5.43 (0.97)	5.18 (1.27)	0.181	5.27 (1.29)	4.80 (1.55)	**0.07**
Orgasm	5.01 (1.31)	4.62 (1.44)	**0.033**	4.66 (1.41)	4.45 (1.61)	0.689
Satisfaction	5.25 (1.08)	5.19 (1.11)	0.713	5.33 (1.13)	4.92 (0.96)	**0.039**
Pain	4.98 (1.55)	4.90 (1.54)	0.624	4.95 (1.56)	4.32 (1.92)	0.157
FSFI_full	30.43 (5.24)	28.88 (6.14)	**0.026**	29.38 (6.08)	27.14 (7.59)	0.229
Menarche	12.22 (1.17)	12.44 (1.34)	0.485	12.43 (1.38)	12.37 (0.95)	0.82
Adrenarche	10.18 (1.14)	10.42 (1.49)	0.637	10.53 (1.45)	9.87 (1.31)	0.16
Age of first intercourse	17.67 (2.00)	17.93 (1.83)	0.242	18.08 (1.89)	17.43 (1.36)	0.171
Number of sex partners	3.79 (3.34)	3.38 (3.95)	0.428	3.67 (4.69)	3.06 (2.46)	0.857

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Discussion

In the present study, a genetic predisposition of female sexual response was revealed. More specifically, T allele (wildtype) of rs2234693 (PvuII) polymorphism of *ERA* gene was correlated with increased levels of arousal and lubrication, whereas A allele (polymorphic) of rs53576 (*OXTR*) polymorphism with increased arousal levels. The concurrence of T allele (wildtype) of *ERA* rs2234693 polymorphism and A allele (polymorphic) of *OXTR* rs53576 polymorphism was correlated with increased arousal and orgasm levels as well as with higher FSFI_full scores. To our knowledge, this is the first study that investigates the interaction between genetic polymorphisms of *ERA* and *OXTR* genes and sexuality parameters.

Our findings are a genetic confirmation of clinical data supporting the cardinal role estrogens play in female sexual activity. In healthy premenopausal women with normal sexual activity who underwent pharmacologically induced hypogonadism, a statistically significant reduction in sexuality parameters has been documented (35). Robust evidence on the prominent role of estrogens in sexual desire are increased levels of libido reported during follicular phase of menstrual cycle as well as in periovulatory period (36, 37). Furthermore, in cases of estrogen deficiency, aggravation of sexual parameters is thoroughly studied. In women with premature ovarian failure (POF), sexual function has been shown to be impaired, especially in the domains of desire and arousal (38). In menopausal period, sexual arousal response is impaired not only because of a decrease in genital vasocongestion and lubrication leading to atrophy of the vaginal epithelium but also because of mood changes from estrogen deprivation and its adverse effects on psychological perception of sexual arousal (39).

Concerning oxytocin, in our study, it was shown that A allele (polymorphic) of rs53576 (*OXTR*) polymorphism was correlated with increased arousal levels. It is well known that *OXTR* is implicated in female sexual response. Based on data from animal studies, female pelvic organs involved in (pre-) copulatory behavior are provided with *OXTR* and oxytocin neural fibers descending into the lumbosacral parts of the spinal cord (40, 41, 42). Peripheral and central effects on the pelvic organs are implicated in the preparation for the copulatory activities with regard to lubrication, muscular contractility and pain suppression for the coming vagino-cervical distension (43, 44, 45, 46, 47, 48, 49, 50).

Studies on humans confirm the presence of *OXTR* on female genital organs indicating a possible 'preparatory role' of oxytocin for the later and final phases of the copulatory process, exerting a direct effect on sensory nerve sensitivity, 'preparing' thus muscular contractions and lubrication effects (51).

Given that there is no functional analysis concerning rs53576 polymorphism, the mechanism by which this polymorphism is implicated in sexual arousal could be elucidated based on data from behavioral traits. It is established that A allele of *OXTR* rs53576 has been associated with decreased hypothalamus and amygdala volumes, which are brain areas that are extensively involved in the regulation of social and emotional behaviors (52, 53). Empathy is defined as the capacity to experience feelings of compassion, warmth and concern in response to other people. Empathy is a complex socio-emotional competency that encompasses components including empathic arousal and empathic concern (54). Individual levels of empathy may be associated with individual differences in unconscious sharing of affect experienced while viewing others in physical distress. Lower scores of empathic concern were associated with A allele of *OXTR* rs53576 polymorphism (55). It has been suggested that empathic tendencies magnify vulnerability for psychological disorders under certain conditions. Increased risk for personal distress and excessive interpersonal guilt in cases of enhanced propensities for empathic sensitivity have been suggested. Personal distress and interpersonal guilt, in turn, contribute to heightened risk for fear/arousal symptoms and anhedonia/misery symptoms, respectively (56). Consequently, it could be presumed that lower scores of empathy could be associated with increased levels of sexual arousal.

The concurrence of T allele (wildtype) of *ERA* rs2234693 polymorphism and A allele (polymorphic) of *OXTR* rs53576 polymorphism was correlated with increased arousal and orgasm levels as well as with higher FSFI_full scores, whereas there was a trend for increased desire levels. It seems that the favorable impact of these alleles on sexuality parameters is synergistic, given that the exclusive presence of T allele of *ERA* rs2234693 was correlated only with increased satisfaction levels compared to the exclusive presence of A allele of *OXTR* rs53576. Interaction between estrogens and oxytonergic system is well established. Estrogens have been shown to increase *OXTR* binding (27), whereas oxytocin has been found to increase expression of ERA (28).

The results of the present study do not seem to be in line with those found in the literature. Neither *ERA*-rs2234693 nor *OXTR*-rs53576 has been shown to account for variability of sexual response. One candidate

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gene study has linked serotonin polymorphisms (*5HT2A*) to reduced sexual desire as a side effect of SSRI medication in 89 adult men and women (57). A further study reported an association between the dopamine D4 receptor gene (*DRD4*) with self-reports of sexual desire and arousal in 52 men and 92 women (58). Interleukin-1beta gene (*IL1B*) has been correlated with variation in vulvar vestibulitis syndrome scores, a broader phenotype for sexual pain symptoms (59). On a genome-wide association study (GWAS) on 2.5 million single-nucleotide polymorphisms, three polymorphic sites (rs13202860, rs1876525 and rs13209281) around 500 kb upstream of the locus *HTR1E* (5-hydroxytryptamine receptor 1E) were associated with arousal (10). Although female sexuality has been associated with *ERB* gene polymorphisms, *ERA* gene polymorphisms have not been related to sexuality parameters (9, 10). Gunst and coworkers reported no association of rs2234693 polymorphism of *ERA* gene with any sexuality parameters (9). This could be attributed to the fact that ERA is involved in sexual behavior via multiple and complicated mechanisms that have not been fully elucidated yet, whereas the mechanisms of ERB action concerning female sexuality seem to be limited. Therefore, a polymorphism of *ERB* gene affecting receptor affinity could be directly related to sexual behavior, while the presence of a single *ERA* polymorphism may not have an impact on sexuality. In fact, estrogen effect on sexuality is the result of *ERA* and *ERB* interaction. Consequently, although single polymorphisms in *ERA* gene may be impotent in exerting an effect on sexual behavior, the importance of ERA in female sexuality is paramount compared to that of ERB, given that *ERA* knockout mice did not display sexual behavior, whereas *ERB* knockout mice exhibited lordosis behavior equal to wildtype ones (60). Our study is the first that finds a relationship between *ERA* gene polymorphism and sexuality parameters. Furthermore, this is the first study that highlights the favorable synergistic impact of wildtype allele of *ERA* gene rs2234693 (T allele) and polymorphic allele of *OXTR* rs53576 (A allele) on sexuality parameters.

However, in drawing overall conclusions, we must take notice of limitations of the current study. The sample is not representative of the general population as it comes from higher education women, whereas sample size is rather small. Another limitation could be the short time span (4 weeks) of FSFI questionnaire. Given that female sexuality is a behavioral phenotype that is quite complex, women's short-term sexual functioning can fluctuate, rendering assessment of sexual functioning speculative. Moreover, the data investigating the association between these polymorphisms and sexual function in women are sparse, whereas candidate gene association studies generate inconclusive results. Failure to replicate genotype studies is common in candidate gene association studies (61). In fact, no genetic study on female sexuality has been successfully replicated (9). Further research and replication of the results are needed to clarify such issues.

In conclusion, female sexuality is a complex behavior that encompasses both biological and psychological components. It seems that endocrine, neurotransmitter and CNS's influences affect parameters of female sexuality characterized by genetic variability due to the presence of a variety of individual genetic polymorphisms such as those concerning *ERA* and *OXTR* genes.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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