BASIC SCIENCE

Implications of the Estrogen Receptor Coactivators SRC1 and SRC2 in the Biological Basis of Gender Incongruence

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ABSTRACT

Introduction: Brain sexual differentiation results from the effects of sex steroids on the developing brain. The presumptive route for brain masculinization is the direct induction of gene expression via activation of the estrogen receptors α and β and the androgen receptor through their binding to ligands and to coactivators, regulating the transcription of multiple genes in a cascade effect.

Aim: To analyze the implication of the estrogen receptor coactivators SRC-1, SRC-2, and SRC-3 in the genetic basis of gender incongruence.

Main Outcome Measures: Analysis of 157 polymorphisms located at the estrogen receptor coactivators SRC-1, SRC-2, and SRC-3, in 94 transgender versus 94 cisgender individuals.

Method: Using SNPStats software, the allele and genotype frequencies were analyzed by $\chi 2$, the strength of the association was measured by binary logistic regression, estimating the odds ratio for each genotype. Measurements of linkage disequilibrium and haplotype frequencies were also performed.

Results: We found significant differences at level $P < .05$ in 8 polymorphisms that correspond to 5.09% of the total. Three were located in SRC-1 and 5 in SRC-2. The odds ratio analysis showed significant differences at level $P < .05$ for multiple patterns of inheritance. The polymorphisms analyzed were in linkage disequilibrium. The SRC-1 haplotypes CGA and CGG (global haplotype association $P < .009$) and the SRC-2 haplotypes GGTAA and GGTAG (global haplotype association $P < .005$) were overrepresented in the transgender population.

Conclusion: The coactivators SRC-1 and SRC-2 could be considered as candidates for increasing the list of potential genes for gender incongruence. **Ramírez KDV, Fernández R, Delgado-Zayas E, et al. Implications of the Estrogen Receptor Coactivators SRC1 and SRC2 in the Biological Basis of Gender Incongruence. Sex Med 2021;9:100368.**

Key Words: Estrogens; Estrogen Coactivators; Gender Dysphoria; Gender Incongruence; SRC-1; SRC-2; SRC-3

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INTRODUCTION

The term gender identity refers to a person's innermost sense of their own gender,[1,2] while sex refers to the biological sex characteristics, based on chromosomal, hormonal, physical, and anatomical characteristics. For clarity in this work, sex will be used interchangeably with natal sex, biological sex, and sex assigned at birth.

Gender identities are classified into "cisgender" and "transgender" umbrellas. Cisgender is used to refer to a gender identity that matches a person's natal sex, while transgender refers to a gender identity that differs from the sex assigned at birth. Gender Incongruence (GI) in the International Classification of Diseases ICD-11[3] and Gender Dysphoria (GD) in the Diagnostic and

Statistical Manual of Mental Disorders DSM-5[4] are characterized by a marked incongruence between one's experienced gender and the sex assigned at birth. In order to meet criteria for the diagnosis of GD, the condition must also be associated with clinically significant distress or impairment in social, occupational, or other important areas of functioning.[3]

The origin of GI[3] appears to be multifactorial. It might be associated with neurodevelopmental processes of the brain[5,6] under the influence of testosterone converted into estradiol in the brain by the action of the aromatase, during a critical period of development. Thus, in mammals, sex differences in the adult brain are established very early in development, when the brain is very immature.[7] In the case of having inherited the SRY gene, during embryogenesis, testosterone secreted by the testes enters the brain and is converted to estradiol by the aromatase.[8] Then, the estradiol acts in the brain by binding to intracellular estrogen receptors located predominantly in neurons, masculinizing specific brain regions.

But a genetic component may also be involved in GI since siblings of transgender individuals are more likely to be transgender, compared with the general population.[9] Most genetic studies that investigate the genetic component of gender incongruence analyze the implication of polymorphisms related to the estrogen receptors (ERs) α and β, the androgen receptor AR or the aromatase CYP19A1,[10–17] as well as the interaction effects (epistasis) between them[18] or the effect of epigenetics.[19,20] This gene selection is based on the fact that sex hormone receptors belong to the nuclear receptor superfamily of ligand activated transcriptional factors. In the case of the AR, their ligand is androgen[21] while for the ER is estrogen, 17β-Estradiol (E_2) in particular.[22] E_2 exerts a wide variety of effects on growth, development, the function of reproductive systems and regulation in the central nervous system.[22,23] The mechanism of action of the two isoforms ERα and ERβ consists of binding with the E_2 ligand to obtain the receptor`s dimerization (Figure 1), originating the necessary conformational changes in the ligand binding domain (LBD)[24] and coupling with the estrogen response elements (EREs)[24] in the genes that are regulated by E_2. On the other hand, this conformational change in the LBD allows coactivators and other coregulating proteins to be recruited. This step is critical for the transcriptional regulation of genes induced by E2[25] (Figure 1).

Among the coactivating proteins are the steroid receptor coactivators (SRCs)[26] that consist of three related members: SRC-1, SRC-2, and SRC-3. The role of SRCs in ER-mediated gene expression was demonstrated by the discovery of increased amplification of the AIB1 gene in ER-positive breast and ovarian cancer cells[27] and partial resistance to E_2[28,29] is evident in knockout mice SRC-1.

Estrogen is produced in many regions of the brain including the hippocampus, the cortex, the cerebellum, the hypothalamus, and the amygdala.[30] The actions of estradiol in the developing brain are generally permanent and range from the establishment

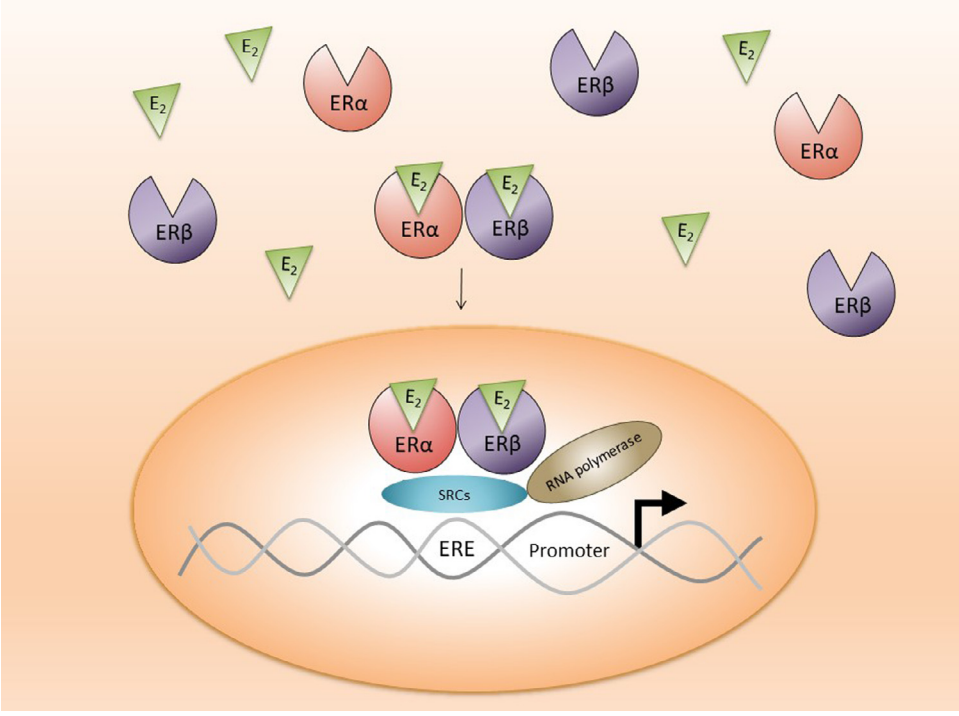

Figure 1. Molecular mechanisms of action of ERs. Hormone 17β-Estradiol (E_2) binds to the nuclear receptor (ERα or ERβ), and after dimerization and translocation to the nucleus, the nuclear receptor complex binds to a specific sequence of DNA known as an estrogen response element (ERE). The nuclear receptor DNA complex in turn recruits the SRCs coactivators that activate the transcription of gene targets.

of sexual differences (cerebral dimorphism), to generalized trophic and neuroprotective effects.[31]

Given the importance of estrogens in brain dimorphism, and the critical role of the coactivators in the transcriptional regulation of genes induced by E_2, and based on previous genetic studies,[10–17] in the present research we analyzed 157 polymorphisms located at SRC-1, SRC-2 and SRC-3 coactivators in a transgender versus a cisgender population. We postulated that variation at the DNA level at the steroid receptor coactivators (SRC-1, SRC-2, and/or SRC-3) could affect the function of the E_2-ER complex, and consequently could also modify the transcription of the genes regulated by E_2.

METHODS AND MATERIALS

Participants

We analyzed 94 transgender individuals (47 transmen and 47 transwomen) versus 94 cis gender individuals (44 cismen and 50 ciswomen). The transgender population was recruited and diagnosed through the Gender Unit of the Clínic Hospital of Barcelona (Spain) at the moment of starting gender-affirming hormonal treatment. All transgender participants identified with the other gender (male or female) and were erotically attracted to persons with the same anatomical sex. All of them presented an early onset of gender nonconformity, before or at puberty, and showed a marked intensity of gender dysphoria. Sexual orientation was established by asking which partner (a man, a woman, both or neither) they would prefer or feel attraction to if they were completely free to choose and their body did not interfere.

The inclusion criteria were: presenting gender incongruence according to ICD-11[3] and having no disorder of sexual development. The exclusion criteria for all participants were DSD (differences in sex development), neurological and hormonal disorder, major medical condition and history of alcohol and/or drug abuse. To rule out the presence of psychiatric disorders and substance abuse in all participants, the Mini-International Neuropsychiatric Interview[32] was administered.

The cisgender population was selected from a country census (Pizarra, Málaga, Spain) matched by geographic origin, race, and sex. The main characteristics of the Pizarra census were described in a previous study.[33]

Written informed consent was obtained from the transgender group after full explanation of the procedures. The DNA samples of the cisgender group were recruited from the biobank generated for the Pizarra study, in which all participants signed informed consent for donation of the samples to the biobank of the Hospital Regional Universitario de Málaga for medical research studies. The study was approved by the UNED Ethics Committee.

DNA Analysis

Genomic DNA was extracted from EDTA blood samples using the DNeasy Blood & Tissue Kit from Qiagen (Madrid, Spain) according to the manufacturer's protocol. The analyzed polymorphisms were single nucleotide polymorphisms (SNPs) located, according to the Ensembl database (www.ensembl.org/), in the steroid receptor coactivators SRC-1, SRC-2, and SRC-3. Genotyping was performed by the array Axiom Spanish BioBank (Affymetrix). Statistical analyses were performed using the free online software SNPStats (http://bioinfo.iconcologia.net/SNPstats).

Statistical Analyses

The analyses were conducted by chromosomic sex, in 2 independent populations: individuals assigned as females at birth and individuals assigned as males at birth, considering significant a P value lower than .05.

The allele and genotype frequencies were analyzed by $\chi 2$ test. The strength of the associations with gender incongruence was measured by binary logistic regression, estimating the odds ratio (OR) for each genotype.

Polymorphisms located very close to each other in the same chromosome tend to be inherited together with a high degree of correlation. This correlation, called linkage disequilibrium, was also analyzed. Moreover, we were also interested in the simultaneous analysis of multiple loci (haplotypes), given that they may themselves be causal variants.[34]

Measurement of linkage disequilibrium, designated as D' and r2, and subsequent measurement of haplotype frequencies were performed using the free online software SNPStats (http://bioinfo.iconcologia.net/SNPstats)[35] using logistic regression models to determine the strength of the associations. In all analyses, a missing value for any response, polymorphism, or covariate was cause for exclusion of that individual from the analysis. Any false positives were controlled with the Bonferroni correction ($P < .05/157 = .0003$). A post-hoc power analysis showed a 67.8% power, with the following study parameters: incidence group (1) 45.74%, incidence group (2) 28.72%. Subjects, group (1): 94. Subjects group (2): 94. Alpha = 0.05.

RESULTS

Analysis of Allele and Genotype Frequencies

We analyzed 157 SNPs distributed in the coactivators SRC-1 (63 SNPs), SRC-2 (64 SNPs), and SRC-3 (30 SNPs) (Table 1) in 94 transgender individuals (47 transmen and 47 transwomen) versus 94 cis gender individuals (44 cismen and 50 ciswomen). All the polymorphisms were in Hardy-Weinberg equilibrium. The prevalence rates for all analyzed polymorphisms were similar to those found in the global 1000 genomes and the European 1000 genomes http://www.1000genomes.org.

When we compared the distribution of the allele frequencies, we found significant differences in 8 SNPs that correspond to 5.09% of the analyzed polymorphisms: three located in SRC-1 (polymorphisms 1, 2, and 3) and five in SRC-2 (polymorphisms

Table 1. Description of the analyzed SRCs

Gene	Chromosome	Location	Function	Analyzed SNPs	SNPs with significant differences
SRC-1	2	24491254-24770702	The protein encoded by this gene acts as a transcriptional coactivator for steroid and nuclear hormone receptors.	63	3
SRC-2	8	70109770-70405390	The encoded protein acts as an intermediary factor for the ligand-dependent activity of nuclear receptors, which regulate their target genes upon binding of cognate response elements.	64	5
SRC-3	20	47501887-47656872	The protein encoded by this gene is a nuclear receptor coactivator that interacts with nuclear hormone receptors to enhance their transcriptional activator functions	30	0
			Total SNPs	157	8
			%	100	5.09

This table shows the main characteristic of the SRCs analyzed: the gene name, the chromosome location, the main function of the protein, the number of SNPs analyzed and the number of the polymorphisms (SNPs) that showed statistical significance $P < .05$.

4 to 8) (Table 1). The description of the statistically significant polymorphisms is in Tables 1 and 2.

Association Analysis of Each Polymorphism With Gender Incongruence

We found significant differences in the analysis of the strength of the association with gender incongruence. The odds ratio (OR) analysis for the P1 to P8 polymorphisms showed significant differences for multiple patterns of inheritance (Table 3):

P1 polymorphism: The genotype T/T was overrepresented in the cis population (OR > 2.13; $P < .034$ for the dominant model).

P2 polymorphism: The genotype T/T was overrepresented in the cis population (OR > 2.12; $P < .014$).

P3 polymorphism: The genotype A/A was overrepresented in the cis population (OR > 2.19; $P < .008$ for the dominant model).

P4 polymorphism: The genotype G/G was overrepresented in the cis population (OR > 2.58; $P < .033$ for the log-additive model).

P5 polymorphism: The G/G genotype was overrepresented in the trans population while the A/G genotype was overrepresented in the cis population (OR > 0.48; $P < .003$ for the codominant model).

P6 polymorphism: The T/T genotype was overrepresented in the trans population while the T/G and G/G were overrepresented in the cis population (OR > 0.42; $P < .007$ for the dominant model).

P7 polymorphism: The A/A genotype was overrepresented in the trans population (OR > 2.42; $P < .024$ recessive model).

P8 polymorphism: The genotype A/A was overrepresented in the cis population (OR > 4.83; $P < .007$ dominant model; OR > 4.64; $P < .006$ log-additive model).

Polymorphism Interaction Analysis With Covariate Sex

When we analyzed the interaction with the covariate sex, only P2 showed significant differences in both trans populations (women and men) (Table 4). The genotype T/G was more

Table 2. Description of the SNPs with significant differences

Gene	Polymorphism	Alias	DNA variation	Regulation by Ensembly	Our study frequency	Global 1000 genomes frequency	European 1000 genomes frequency
SRC-1	rs10495747	P1	T/C	Intron Variant	C= 0.11	C=0.1330	C=0.1153
	rs2584940	P2	T/G	Regulatory Region Variant/ Intron Variant	G= 0.38	G=0.4605	G=0.4125
	rs6756785	P3	A/G	Intergenic Variant/ 500B Downstream Variant	G=0.32	G=0.2115	G=0.2883
SRC-2	rs76968380	P4	G/A	Intergenic Variant	A=0.06	A=0.1138	A=0.0646
	rs34406737	P5	G/A	Intergenic Variant	A=0.15	A=0.1300	A=0.1262
	rs1963250	P6	G/T	Intergenic Variant	T=0.57	T=0.5691	T=0.5368
	rs10755950	P7	G/A	Intergenic Variant	A=0.42	A=0.5655	A=0.4483
	rs56055423	P8	A/G	Intergenic Variant	G=0.05	G=0.0132	G=0.0457

This table shows the main characteristic of the polymorphisms that showed statistical significance: the gene name, the polymorphism name, the alias used in this work, the DNA variation, the gene location of the polymorphisms, and the frequencies of the polymorphisms in our study versus the Global 1000 genomes and the European 1000 genomes databases.

Table 3. Polymorphism association analysis with gender incongruence, in different models of inheritance (n = 188, crude analysis)

Model	Genotype	Cis groups (%)	Trans groups (%)	OR	*P*	AIC	BIC
P1 polymorphism (rs10495747)							
Codominant	T/T	79 (84%)	67 (71.3%)	**1.00 (reference)**	.07	263.1	276
	T/C	15 (16%)	26 (27.7%)	2.05 (1.00–4.19)			
	C/C	0 (0%)	1 (1.1%)	NA (0.00–NA)			
Dominant	T/T	79 (84%)	67 (71.3%)	**1.00 (reference)**	.034*	261.9	271.7
	T/C-C/C	15 (16%)	27 (28.7%)	2.13 (1.05–4.33)			
Recessive	T/T-T/C	94 (100%)	93 (98.9%)	**1.00 (reference)**	.25	265.1	274.8
	C/C	0 (0%)	1 (1.1%)	NA (0.00–NA)			
Overdominant	T/T-C/C	79 (84%)	68 (72.3%)	**1.00 (reference)**	.05*	262.6	272.3
	T/C	15 (16%)	26 (27.7%)	2.02 (0.99–4.13)			
Log-additive	—	—	—	2.15 (1.07–4.30)	.027*	261.5	271.3
P2 polymorphism (rs2584940)							
Codominant	T/T	43 (45.7%)	27 (28.7%)	**1.00 (reference)**	.039*	261.9	274.9
	T/G	42 (44.7%)	52 (55.3%)	2.00 (1.06–3.76)			
	G/G	9 (9.6%)	15 (16%)	2.72 (1.04–7.10)			
Dominant	T/T	43 (45.7%)	27 (28.7%)	**1.00 (reference)**	.014*	260.4	270.1
	T/G-G/G	51 (54.3%)	67 (71.3%)	2.12 (1.16–3.89)			
Recessive	T/T-T/G	85 (90.4%)	79 (84%)	**1.00 (reference)**	.18	264.6	274.3
	G/G	9 (9.6%)	15 (16%)	1.82 (0.75–4.39)			
Overdominant	T/T-G/G	52 (55.3%)	42 (44.7%)	**1.00 (reference)**	.14	264.3	274
	T/G	42 (44.7%)	52 (55.3%)	1.54 (0.87–2.74)			
Log-additive	—	—	—	1.74 (1.11–2.73)	.013*	260.3	270
P3 polymorphism (rs6756785)							
Codominant	A/A	50 (53.2%)	32 (34%)	**1.00 (reference)**	.03*	261.4	274.4
	A/G	38 (40.4%)	54 (57.5%)	2.21 (1.20–4.06)			
	G/G	6 (6.4%)	8 (8.5%)	2.09 (0.66–6.60)			
Dominant	A/A	50 (53.2%)	32 (34%)	**1.00 (reference)**	.008*	259.4	269.2
	A/G-G/G	44 (46.8%)	62 (66%)	2.19 (1.22–3.95)			
Recessive	A/A-A/G	88 (93.6%)	86 (91.5%)	**1.00 (reference)**	.57	266.1	275.8
	G/G	6 (6.4%)	8 (8.5%)	1.38 (0.46–4.14)			
Overdominant	A/A-G/G	56 (59.6%)	40 (42.5%)	**1.00 (reference)**	.02*	261	270.8
	A/G	38 (40.4%)	54 (57.5%)	1.98 (1.11–3.54)			
Log-additive	—	—	—	1.77 (1.10–2.87)	.018*	260.8	270.5
P4 polymorphism (rs76968380)							
Codominant	G/G	88 (93.6%)	80 (85.1%)	**1.00 (reference)**	.073	263.2	276.1
	A/G	6 (6.4%)	12 (12.8%)	2.22 (0.79–6.19)			
	A/A	0 (0%)	2 (2.1%)	NA (0.00–NA)			
Dominant	G/G	88 (93.6%)	80 (85.1%)	**1.00 (reference)**	.054	262.7	272.4

(continued)

Table 3. Continued

Model	Genotype	Cis groups (%)	Trans groups (%)	OR	*P*	AIC	BIC
	A/G-A/A	6 (6.4%)	14 (14.9%)	2.58 (0.95–7.05)			
Recessive	G/G-A/G	94 (100%)	92 (97.9%)	**1.00 (reference)**	.095	263.6	273.3
	A/A	0 (0%)	2 (2.1%)	NA (0.00–NA)			
Overdominant	G/G-A/A	88 (93.6%)	82 (87.2%)	**1.00 (reference)**	.13	264.1	273.9
	A/G	6 (6.4%)	12 (12.8%)	2.16 (0.77–6.03)			
Log-additive	—	—	—	2.58 (1.01–6.60)	.033*	261.9	271.6
P5 polymorphism (rs34406737)							
Codominant	G/G	63 (67%)	72 (76.6%)	**1.00 (reference)**	.003*	256.9	269.9
	A/G	31 (33%)	17 (18.1%)	0.48 (0.24–0.95)			
	A/A	0 (0%)	5 (5.3%)	NA (0.00–NA)			
Dominant	G/G	63 (67%)	72 (76.6%)	**1.00 (reference)**	.15	264.4	274.1
	A/G-A/A	31 (33%)	22 (23.4%)	0.63 (0.33–1.19)			
Recessive	G/G-A/G	94 (100%)	89 (94.7%)	**1.00 (reference)**	.008*	259.4	269.1
	A/A	0 (0%)	5 (5.3%)	NA (0.00–NA)			
Overdominant	G/G-A/A	63 (67%)	77 (81.9%)	**1.00 (reference)**	.02*	261	270.7
	A/G	31 (33%)	17 (18.1%)	0.45 (0.23–0.89)			
Log-additive	—	—	—	0.86 (0.49–1.49)	.58	266.1	275.8
P6 polymorphism (rs1963250)							
Codominant	T/T	20 (21.3%)	37 (39.4%)	**1.00 (reference)**	.016*	260.2	273.1
	T/G	54 (57.5%)	46 (48.9%)	0.46 (0.24–0.91)			
	G/G	20 (21.3%)	11 (11.7%)	0.30 (0.12–0.75)			
Dominant	T/T	20 (21.3%)	37 (39.4%)	**1.00 (reference)**	.007*	259.2	268.9
	T/G-G/G	74 (78.7%)	57 (60.6%)	0.42 (0.22–0.80)			
Recessive	T/T-T/G	74 (78.7%)	83 (88.3%)	**1.00 (reference)**	.079	263.3	273.1
	G/G	20 (21.3%)	11 (11.7%)	0.49 (0.22–1.10)			
Overdominant	T/T-G/G	40 (42.5%)	48 (51.1%)	**1.00 (reference)**	.25	265.1	274.8
	T/G	54 (57.5%)	46 (48.9%)	0.71 (0.40–1.27)			
Log-additive	—	—	—	0.53 (0.34–0.83)	.005*	258.5	268.2
P7 polymorphism (rs10755950)							
Codominant	G/G	33 (35.1%)	31 (33%)	**1.00 (reference)**	.068	263.1	276
	A/G	50 (53.2%)	40 (42.5%)	0.85 (0.44–1.61)			
	A/A	11 (11.7%)	23 (24.5%)	2.19 (0.91–5.27)			
Dominant	G/G	33 (35.1%)	31 (33%)	**1.00 (reference)**	.79	266.4	276.1
	A/G-A/A	61 (64.9%)	63 (67%)	1.08 (0.59–1.99)			
Recessive	G/G-A/G	83 (88.3%)	71 (75.5%)	**1.00 (reference)**	.024*	261.3	271
	A/A	11 (11.7%)	23 (24.5%)	2.42 (1.10–5.33)			
Overdominant	G/G-A/A	44 (46.8%)	54 (57.5%)	**1.00 (reference)**	.14	264.3	274
	A/G	50 (53.2%)	40 (42.5%)	0.65 (0.36–1.15)			

(continued)

Table 3. Continued

Model	Genotype	Cis groups (%)	Trans groups (%)	OR	*P*	AIC	BIC
Log-additive	—	—	—	1.34 (0.89–2.03)	.16	264.4	274.2
P8 polymorphism (rs56055423)							
Codominant	A/A	91 (96.8%)	81 (86.2%)	**1.00 (reference)**	.023*	260.8	273.8
	A/G	3 (3.2%)	12 (12.8%)	4.44 (1.20–16.44)			
	G/G	0 (0%)	1 (1.1%)	NA (0.00-NA)			
Dominant	A/A	91 (96.8%)	81 (86.2%)	**1.00 (reference)**	.007*	259.3	269
	A/G-G/G	3 (3.2%)	13 (13.8%)	4.83 (1.32–17.67)			
Recessive	A/A-A/G	94 (100%)	93 (98.9%)	**1.00 (reference)**	.23	265	274.7
	G/G	0 (0%)	1 (1.1%)	NA (0.00-NA)			
Overdominant	A/A-G/G	91 (96.8%)	82 (87.2%)	**1.00 (reference)**	.014*	260.4	270.1
	A/G	3 (3.2%)	12 (12.8%)	4.41 (1.19–16.33)			
Log-additive	—	—	—	4.64 (1.30–16.56)	.006*	259	268.7

Association analysis between polymorphisms and gender incongruence. The table shows the estimation of the OR (odds ratio) for each genotype with respect to the reference genotype (1.00 (reference)) in different inheritance models (codominant, dominant, recessive, overdominant and log-additive. AIC = Akaike's information criterion; BIC = Bayesian information criterion; OR = odds ratio.
*Statistically significant ($P < .05$).

frequent in trans people with female natal sex than in the cis female population (OR > 2.76; P < .029) while the genotype G/G was more frequent in trans people with male natal sex than in the cis male population (OR>8.0; P < .016).

The other polymorphisms (P3, P5, P6, and P8) showed significant differences in the distribution of the genotypes only in the population with male natal sex (Table 4), while the P5 showed the significant difference only in the female natal sex.

Haplotype Analysis

For polymorphisms located in SRC-1 (Table 5), the T allele for P1 was linked to the T allele for P2, and to the A allele for P3 (haplotype 1: T-T-A) with a total frequency of 0.45 (Table 5). However, the C allele for P1 was linked to the G allele for P2 and to the A allele for P3 (haplotype 5: C-G-A) (OR > 2.62; $P < .05$) or to the G allele for P3 (haplotype 7: C-G-G) ($P < .0001$) (Table 5), with a global haplotype association P < 0.009.

For polymorphisms located in SRC-2 (Table 6), the significant haplotypes were haplotype 2: (G-G-T-A-A) (OR > 2.49; $P < .02$), and the haplotype 8: (G-G-T-A-G) (OR > 12.86; $P < .028$) (Table 6), with a global haplotype association $P < .005$.

DISCUSSION

The estrogen receptors α and β, in addition to hormonal receptors, are also transcription factors that, when exposed to their ligand, dimerize and form complexes, binding to coactivator proteins[36] (Figure 1), modifying the transcription of multiple target genes in cascade mode[37] and, ultimately, the neuronal function. Therefore, coactivators are proteins that influence the ability of the transcription factors to activate or inhibit expression of multiple genes.[26] Some coactivators for sex steroids receptors are SRC-1, SRC-2, and SRC-3.[23] Given the intimate relationship between sex steroids and brain dimorphism, and sex steroids and gene transcription, it seems is clear that we can to postulate the implication of the DNA coactivators in the process of brain dimorphism.

In our study, we analyzed the allele and genotype frequencies, the association with gender incongruence, the interactions with the covariate sex, and the linkage disequilibrium of 157 polymorphisms located at the coactivators SRC-1, SRC-2, and SRC-3 in a population of 94 transgender individuals versus 94 cis gender individuals. We found significant differences in eight polymorphisms located in SRC-1 and SRC-2. Furthermore, only P2 (rs2584940) in SRC-1 showed significant differences in the interaction analysis with covariate "sex". When the analysis was carried out in separate populations according to their natal sex, we found that polymorphisms P2 and P5 were statistically significant for female natal sex populations, while P2, P3, P6, and P8 were statistically significant for male natal sex populations (Table 4). The SRC-1 haplotypes C-G-A and C-G-G and the

Table 4. P2, P3, P5, P6 and P8 interaction analysis with covariate sex. The table shows the ORs (odds ratios) for the P2, P3, P5, P6 and P8 polymorphism interactions with the covariable sex. First comparing assigned female at birth (cisgender vs. transgender individuals), and assigned male at birth (cisgender vs. transgender individuals), the comparisons of the "covariate sex within each polymorphism", and finally each "polymorphism within the covariate sex".

P2 and covariate sex cross-classification interaction table (n = 188, crude analysis)

	Female				Male			
Genotypes	Cis population	Trans population	OR (95% CI)	*P*	Cis population	Trans population	OR (95% CI)	*P*
T/T	22	11	1	—	21	16	1.52 (0.58-4.03)	.404
T/G	21	29	2.76 (1.10–6.90)	.029*	21	23	2.19 (0.86-5.58)	.100
G/G	7	7	2.00 (0.56–7.15)	.289	2	8	8.00 (1.45-44.24)	.016*
Interaction *P*-value: 0.23								
Sex within P2 (n = 188, crude analysis)								
Genotypes	Sex	Cis population	Trans population	OR (95% CI)	*P*			
T/T	female	22	11	1	—			
	male	21	16	1.52 (0.58–4.03)	.404			
T/G	female	21	29	1	—			
	male	21	23	0.79 (0.35–1.79)	.583			
G/G	female	7	7	1	—			
	male	2	8	4.00 (0.62–25.96)	.146			
Test for interaction in the trend: 0.76								
P2 within sex (n = 188, crude analysis)								
Sex	Genotypes	Cis population	Trans population	OR (95% CI)	*P*			
	T/T	22	11	1	—			
female	T/G	21	29	2.76 (1.10–6.90)	.029*			
	G/G	7	7	2.00 (0.56–7.15)	.289			
	T/T	21	16	1	—			
male	T/G	21	23	1.44 (0.60–3.46)	.422			
	G/G	2	8	5.25 (0.98–28.18)	.052			
Test for interaction in the trend: 0.23								

P3 and sex cross-classification interaction table (n = 188, crude analysis)

	Female				Male			
Genotypes	Cis population	Trans population	OR (95% CI)	*P*	Cis population	Trans population	OR (95% CI)	*P*
A/A	27	17	1	—	23	15	1.04 (0.43–2.52)	.936
A/G	19	26	2.17 (0.93–5.07)	.072	19	28	2.34 (1.01–5.43)	.046*
G/G	4	4	1.59 (0.35–7.21)	.559	2	4	3.18 (0.52–19.27)	.211

(continued)

Table 4. Continued

P3 and sex cross-classification interaction table (n = 188, crude analysis)

	Female				Male			
Genotypes	Cis population	Trans population	OR (95% CI)	*P*	Cis population	Trans population	OR (95% CI)	*P*
Test for interaction in the trend: 0.64								
Sex within P3 (n = 188, crude analysis)								
Genotypes	Sex	Cis population	Trans population	OR (95% CI)	*P*			
A/A	female	27	17	1	—			
	male	23	15	1.04 (0.43–2.52)	.936			
A/G	female	19	26	1	—			
	male	19	28	1.08 (0.47–2.47)	.865			
G/G	female	4	4	1	—			
	male	2	4	2.00 (0.22–17.89)	.547			
Test for interaction in the trend: 0.64								
P3 within sex (n = 188, crude analysis)								
Sex	Genotypes	Cis population	Trans population	OR (95% CI)	*P*			
female	A/A	27	17	1	—			
	A/G	19	26	2.17 (0.93–5.07)	.072			
	G/G	4	4	1.59 (0.35–7.21)	.559			
male	A/A	23	15	1	—			
	A/G	19	28	2.26 (0.94–5.41)	.067			
	G/G	2	4	3.07 (0.50–18.89)	.228			
Test for interaction in the trend: 0.86								

P5 and sex cross-classification interaction table (n=188, crude analysis)

	Female				Male			
Genotypes	Cis population	Trans population	OR (95% CI)	*P*	Cis population	Trans population	OR (95% CI)	*P*
G/G	29	38	1	—	34	34	0.76 (0.39–1.50)	.432
A/G	21	7	0.25 (0.10–0.68)	.004*	10	10	0.76 (0.28–2.08)	.603
A/A	0	2	—	—	0	3	—	—
Interaction *P*-value: 0.15								
Sex within P5 (n = 188, crude analysis)								
Genotypes	Sex	Cis population	Trans population	OR (95% CI)	*P*			
G/G	female	29	38	1	—			

(continued)

Table 4. Continued

P5 and sex cross-classification interaction table (n=188, crude analysis)

	Female				Male			
Genotypes	Cis population	Trans population	OR (95% CI)	*P*	Cis population	Trans population	OR (95% CI)	*P*
	male	34	34	0.76 (0.39–1.50)	.432			
A/G	female	21	7	1	—			
	male	10	10	3.00 (0.88–10.21)	.078			
A/A	female	0	2	1	—			
	male	0	3	1	—			
Test for interaction in the trend: 0.044*								
P5 within sex (n = 188, crude analysis)								
Sex	Genotypes	Cis population	Trans population	OR (95% CI)	*P*			
female	G/G	29	38	1	—			
	A/G	21	7	0.25 (0.10–0.68)	.004*			
	A/A	0	2	—	—			
male	G/G	34	34	1	—			
	A/G	10	10	1.00 (0.37–2.71)	1			
	A/A	0	3	—	—			
Test for interaction in the trend: 0.15								

P6 and sex cross-classification interaction table (n=188, crude analysis)

	Female				Male			
Genotypes	Cis population	Trans population	OR (95% CI)	*P*	Cis population	Trans population	OR (95% CI)	*P*
T/T	10	15	1	—	10	22	1.47 (0.49–4.38)	.500
T/G	31	23	0.49 (0.19–1.30)	.146	23	23	0.67 (0.25–1.79)	.433
G/G	9	9	0.67 (0.20–2.26)	.528	11	2	0.12 (0.02–0.67)	.017*
Interaction *P*-value: .074								
Sex within P6 (n = 188, crude analysis)								
Genotypes	Sex	Cis population	Trans population	OR (95% CI)	*P*			
T/T	female	10	15	1	—			
	male	10	22	1.47 (0.49–4.38)	.500			
T/G	female	31	23	1	—			
	male	23	23	1.35 (0.61–2.97)	.466			

(continued)

Table 4. Continued

P6 and sex cross-classification interaction table (n=188, crude analysis)

	Female				Male			
Genotypes	Cis population	Trans population	OR (95% CI)	*P*	Cis population	Trans population	OR (95% CI)	*P*
G/G	female	9	9	1	—			
	male	11	2	0.18 (0.03–1.06)	.058			
Test for interaction in the trend: 0.072								
P6 within sex (n = 188, crude analysis)								
Sex	**Genotypes**	**Cis population**	**Trans population**	**OR (95% CI)**	***P***			
female	T/T	10	15	1	—			
	T/G	31	23	0.49 (0.19–1.30)	.146			
	G/G	9	9	0.67 (0.20–2.26)	.528			
male	T/T	10	22	1	—			
	T/G	23	23	0.45 (0.18–1.17)	.094			
	G/G	11	2	0.08 (0.02–0.44)	.001*			
Test for interaction in the trend: 0.074								

P8 and sex cross-classification interaction table (n = 188, crude analysis)

	Female				Male			
Genotypes	Cis population	Trans population	OR (95% CI)	*P*	Cis population	Trans population	OR (95% CI)	*P*
A/A	49	43	1	—	42	38	1.03 (0.57–1.88)	.929
A/G	1	3	3.42 (0.34–34.10)	.299	2	9	5.13 (1.05–25.05)	.042*
G/G	0	1	—	—	0	0	—	—
Interaction *P*-value: .8								
Sex within P8 (n = 188, crude analysis)								
Genotypes	**Sex**	**Cis population**	**Trans population**	**OR (95% CI)**	***P***			
A/A	female	49	43	1	—			
	male	42	38	1.03 (0.57–1.88)	.929			
A/G	female	1	3	1	—			
	male	2	9	1.50 (0.10–23.07)	.782			
G/G	female	0	1	1	—			
	male	0	0	—	—			
Test for interaction in the trend: 0.89								

(continued)

Table 4. Continued

P8 and sex cross-classification interaction table (n = 188, crude analysis)								
	Female				Male			
Genotypes	Cis population	Trans population	OR (95% CI)	P	Cis population	Trans population	OR (95% CI)	P
P8 within sex (n = 188, crude analysis)								
Sex	Genotypes	Cis population	Trans population	OR (95% CI)	P			
female	A/A	49	43	1	—			
	A/G	1	3	3.42 (0.34–34.10)	.299			
	G/G	0	1	—	—			
male	A/A	42	38	1	—			
	A/G	2	9	4.97 (1.01–24.48)	.048*			
	G/G	0	0	—	—			
Test for interaction in the trend: .8								

*Statistically significant (P < .05).

SRC-2 haplotypes G-G-T-A-A and G-G-T-A-G were more frequent in the trans population and showed statistical significance.

Based on experiments in rodents, it is believed that male sexual differentiation of the brain is caused by androgens, after conversion to estrogens by the aromatase. Moreover, observations in human subjects show that the direct effects of testosterone on the developing fetal brain and also during puberty, are of great importance for the development of male gender identity.[38] However, in the analysis that our group carried out on androgen coactivators (NCOA-4), we did not find any significant differences (data not shown).

Our data are in concordance with recent work that showed that the nuclear receptor coactivators, SRC-1 and SRC-2, are essential for efficient ER transcriptional activity in brain.[39] Furthermore, SRC-1 and SRC-2 are distributed in several specific areas of the brain in different proportions, such as the hypothalamus and the hippocampus, showing at the same time, differences in the binding preference to ER α or β subtypes when located in different brain regions.[39,40] For example, SRC-1 from the hippocampus interacts equally with ERα and ERβ, while SRC-1 obtained from hypothalamus interacts more with ERα than with ERβ. On the other hand, SRC-2 interacts with ERα in the hippocampus but, in contrast, did not interact with ERβ under any ligand condition.

These differential interactions of SRC-1 and SRC-2 with the ER subtypes suggest that these brain regions have distinct expression patterns of coregulators. Understanding how nuclear receptor coactivators' function with various steroid receptors is critical to understanding how the hormones act in different brain regions.

Moreover, our results are also in concordance with the study of the functional significance of the nuclear receptor coactivator SRC-1 in the developing brain.[41] The authors, Auger et al, investigated the consequence of reducing SRC-1 protein during sexual differentiation of the brain, and reported that reducing this protein interferes with the defeminizing actions of estrogen in neonatal rat brains. Their data indicated that SRC-1 expression is critically involved in the hormone-dependent development of normal male reproductive behavior and brain morphology.

Consequently, our data are in agreement with the results of Auger et al,[41] since the polymorphic analysis of this coactivator showed significant differences when allelic and genotypic frequencies were analyzed. Furthermore, the P2 polymorphism, located in SRC-1, also showed statistically significant interactions with the covariate "sex."

One of the limitations of our study is that the sample analyzed is small and not all P values pass the Bonferroni correction. To make our study more robust, it would be necessary to analyze a larger sample, or validate the conclusions with a new analysis from another trans population.

In conclusion, our results have shown that the coactivators, SRC-1 and SRC-2 could be considered as candidates for increasing the list of potential "susceptibility" genes for gender incongruence. Furthermore, our data continue to support the hypothesis that gender incongruence is a multifactorial complex trait, involving intricate

Table 5. Haplotype analysis for polymorphisms located in SRC-1 (P1, P2 and P3 polymorphisms)

Haplotype frequencies estimation and haplotype association with response (n = 188, adjusted by sex)									
Haplotypes	P1	P2	P3	Total	Cis population	Trans population	Cumulative frequency	OR (95% CI)	*P*-value
1	T	T	A	0.4501	0.5201	0.377	0.4501	**1.00 (reference)**	—
2	T	T	G	0.1495	0.134	0.1699	0.5996	2.25 (0.99–5.13)	.054
3	T	G	G	0.147	0.1319	0.1601	0.7466	1.73 (0.81–3.71)	.16
4	T	G	A	0.139	0.1341	0.144	0.8856	1.80 (0.81–3.97)	.15
5	C	G	A	0.069	0.0531	0.0897	0.9546	2.62 (1.00–6.83)	.05*
6	C	T	A	0.0228	0.0267	0.0169	0.9774	1.39 (0.23–8.34)	.72
7	C	G	G	0.0226	0	0.0423	1	379142884.10 (379142883.16–379142885.04)	<.0001*,†
Global haplotype association *P*-value: .009*									

The risk for each haplotype is compared with regards to the reference category (1.00 reference); OR = Odds ratio.
*Statistically significant ($P < .05$).
†Statistically significant after Bonferroni correction ($P < .05/157 = .0003$).

Table 6. Haplotype analysis for polymorphisms located in SRC-2 (P4, P5, P6, P7 and P8 polymorphisms)

Haplotype frequencies estimation and haplotype association with response (n = 188, adjusted by sex)										
Haplotypes	P4	P5	P6	P7	P8	Total	Cis population	Trans population	Cumulative frequency	OR (95% CI)	*P*-value
1	G	G	G	G	A	0.2546	0.3239	0.1963	0.2546	**1.00 (reference)**	—
2	G	G	T	A	A	0.2206	0.2142	0.236	0.4752	2.49 (1.16–5.34)	.02*
3	G	G	T	G	A	0.2022	0.1721	0.2303	0.6773	2.00 (0.93–4.31)	.079
4	G	G	G	A	A	0.0891	0.0861	0.0793	0.7664	1.05 (0.38–2.88)	.92
5	G	A	G	G	A	0.0474	0.0656	0.0235	0.8138	0.55 (0.11–2.89)	.48
6	G	A	T	A	A	0.0419	0.0474	0.0423	0.8557	1.11 (0.24–5.24)	.89
7	G	A	T	G	A	0.041	0.0393	0.0426	0.8967	3.00 (0.10–86.09)	.52
8	G	G	T	A	G	0.0217	0	0.036	0.9184	12.86 (1.34–123.38)	.028*
9	A	G	T	G	A	0.0173	0.0085	0.0228	0.9357	5.62 (0.56–56.71)	.15
10	A	G	G	G	A	0.0165	0.0077	0.0205	0.9522	3.53 (0.40–31.50)	.26
11	G	A	T	A	G	0.0138	0.0028	0.0189	0.9659	2.68 (0.27–26.20)	.4
12	A	G	T	A	A	0.0108	0.0158	0.0093	0.9767	0.00 (-Inf–Inf)	1
rare	*	*	*	*	*				1	2688767424666564139 4185292538194458249 5847650337136368439 78752.00 (268876742466 2284679936719703712 0 80660307679137154433 6 569204736.00 - 26887674 24670843598900338803 9 26810989609273869588 2 937118752768.00)	<.0001*,†
Global haplotype association *P*-value: .005*											

The risk for each haplotype is compared with regards to the reference category (1.00 reference); OR = Odds ratio.
*Statistically significant ($P < .05$).
†Statistically significant after Bonferroni correction ($P < .05/157 = .0003$).

interactions among sex steroids, sex steroids receptors, coactivators, and multiple other genes and polymorphisms.

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REFERENCES

1. Human Rights Campaign. Glossary of terms. http://www.hrc.org/resources/glossary-of-terms. n.d.
2. Gómez-Gil E. Disforia de género. Man Sexol Clín Editorial Méd Panamericana; 2019, p. 400–2.
3. World Health Organization. The ICD-11. International classification of diseases for mortality and morbidity statistics eleventh revision. 2018. https://doi.org/https://icd.who.int/.
4. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Washington, DC: DSM-5; 2013.
5. Guillamón A, Junqué C, Gómez-Gil E. A review of the status of brain structure research in transsexualism. **Arch Sex Behav 2016;45:1615–1648 https://doi.org/http://dx.doi.org/10.1007/s10508-016-0768-5.**
6. Nguyen HB, Loughead J, Lipner E, et al. What has sex got to do with it? The role of hormones in the transgender brain. **Neuropsychopharmacology 2019;44:22–37 https://doi.org/10.1038/s41386-018-0140-7.**
7. McCarthy MM. A new view of sexual differentiation of mammalian brain. **J Comp Physiol A Neuroethol Sens Neural Behav Physiol 2019 https://doi.org/10.1007/s00359-019-01376-8.**
8. Balthazart J, Ball GF. New insights into the regulation and function of brain estrogen synthase (aromatase). **Trends Neurosci 1998;21:243–249.**
9. Gómez-Gil E, Esteva I, Almaraz MC, et al. Familiality of gender identity disorder in non-twin siblings. **Arch Sex Behav 2010;39:546–552 https://doi.org/10.1007/s10508-009-9524-4.**
10. Henningsson S, Westberg L, Nilsson S, et al. Sex steroid-related genes and male-to-female transsexualism. **Psychoneuroendocrinology 2005;30:657–664 https://doi.org/10.1016/j.psyneuen.2005.02.006.**
11. Hare L, Bernard P, Sánchez FJJ, et al. Androgen receptor repeat length polymorphism associated with male-to-female transsexualism. **Biol Psychiatry 2009;65:93–96 https://doi.org/http://dx.doi.org/10.1016/j.biopsych.2008.08.033.**
12. Ujike H, Otani K, Nakatsuka M, et al. Association study of gender identity disorder and sex hormone-related genes. **Prog Neuropsychopharmacol Biol Psychiatry 2009;33:1241–1244 https://doi.org/http://dx.doi.org/10.1016/j.pnpbp.2009.07.008.**
13. Fernández R, Esteva I, Gómez-Gil E, et al. The (CA)n polymorphism of ERβ gene is associated with FtM transsexualism. **J Sex Med 2014;11:720–728 https://doi.org/http://dx.doi.org/10.1111/jsm.12398.**
14. Foreman M, Hare L, York K, et al. Genetic link between gender dysphoria and sex hormone signaling. **J Clin Endocrinol Metab 2019;104:390–396 https://doi.org/10.1210/jc.2018-01105.**
15. Fernández R, Esteva I, Gómez-Gil E, et al. Association study of ERβ, AR, and CYP19A1 genes and MtF transsexualism. **J Sex Med 2014;11:2986–2994 https://doi.org/http://dx.doi.org/10.1111/jsm.12673.**
16. Cortés-Cortés J, Fernández R, Teijeiro N, et al. Genotypes and haplotypes of the estrogen receptor α gene (ESR1) are associated with female-to-male gender dysphoria. **J Sex Med 2017;14:464–472 https://doi.org/10.1016/j.jsxm.2016.12.234.**
17. Fernández R, Delgado-Zayas E, Ramírez K, et al. Analysis of four polymorphisms located at the promoter of the estrogen receptor alpha ESR1 gene in a population with gender incongruence. **Sex Med 2020;8:490–500 https://doi.org/10.1016/j.esxm.2020.04.002.**
18. Fernández R, Guillamon A, Cortés-Cortés J, et al. Molecular basis of gender dysphoria: Androgen and estrogen receptor interaction. **Psychoneuroendocrinology 2018;98:161–167 https://doi.org/10.1016/j.psyneuen.2018.07.032.**
19. Fernández R, Ramírez K, Gómez-Gil E, et al. Gender-affirming hormone therapy modifies the CpG methylation pattern of the ESR1 gene promoter after six months of treatment in transmen. **J Sex Med 2020;17:1795–1806 https://doi.org/10.1016/j.jsxm.2020.05.027.**
20. Aranda G, Fernández-Rebollo E, Pradas-Juni M, et al. Effects of sex steroids on the pattern of methylation and expression of the promoter region of estrogen and androgen receptors in people with gender dysphoria under cross-sex hormone treatment. **J Steroid Biochem Mol Biol 2017;172:20–28 https://doi.org/10.1016/j.jsbmb.2017.05.010.**
21. Wåhlin-Jacobsen S, Flanagan JN, Pedersen AT, et al. Androgen receptor polymorphism and female sexual function and desire. **J Sex Med 2018;15:1537–1546 https://doi.org/10.1016/j.jsxm.2018.09.013.**
22. Métivier R, Penot G, Hübner MR, et al. Estrogen receptor-alpha directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. **Cell 2003;115:751–763 https://doi.org/10.1016/s0092-8674(03)00934-6.**
23. Klinge CM. Estrogen receptor interaction with co-activators and co-repressors. **Steroids 2000;65:227–251 https://doi.org/10.1016/s0039-128x(99)00107-5.**

24. Nilsson S, Mäkelä S, Treuter E, et al. Mechanisms of estrogen action. **Physiol Rev 2001;81:1535–1565 https://doi.org/10.1152/physrev.2001.81.4.1535.**
25. Kenna NJMC, Lanz RB, Malley BWO. Nuclear receptor coregulators: Cellular and molecular biology. **Endocr Rev 2015;20:321–344 https://doi.org/10.1210/edrv.20.3.0366.**
26. Eyster KM. The estrogen receptors : An overview from different perspectives. **Methods Mol Biol 2016;1366:1–10 https://doi.org/10.1007/978-1-4939-3127-9.**
27. Xu J, Qiu Y, DeMayo FJ, et al. Partial hormone resistance in mice with disruption of the steroid eceptor coactivator-1 (SRC-1) gene. **Science 1998;279:1922–1925 https://doi.org/10.1126/science.279.5358.1922.**
28. Sanyal A, Kearns ANNE, Sibonga JD, et al. Effects of loss of steroid receptor coactivator-1 on the skeletal response to estrogen in mice. **Endocrinology 2004;145:913–921 https://doi.org/10.1210/en.2003-1089.**
29. Mödder U, Sanyal A, Xu J, et al. The skeletal response to estrogen is impaired in female but not in male steroid receptor coactivator (SRC)-1 knock out mice. **Bone 2008;42:414–421 https://doi.org/10.1016/j.bone.2007.10.017.**
30. Cui J, Shen Y, Li R. Estrogen synthesis and signaling pathways during aging: from periphery to brain. **Trends Mol Med 2013;19:197–209 https://doi.org/10.1016/j.molmed.2012.12.007.**
31. McCarthy MM. The two faces of estradiol: effects on the developing brain. **Neuroscientist 2009;15:599–610 https://doi.org/10.1177/1073858409340924.**
32. Sheehan D V, Lecrubier Y, Sheehan KH, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. **J Clin Psychiatry 1998;59 (Suppl 20):22–33 quiz 34-57.**
33. Soriguer F, Gutiérrez-Repiso C, Rubio-Martín E, et al. Metabolically healthy but obese, a matter of time? Findings from the prospective Pizarra study. **J Clin Endocrinol Metab 2013;98:2318–2325 https://doi.org/http://dx.doi.org/10.1210/jc.2012-4253.**
34. Stram D. Multi-SNP haplotype analysis methods for association analysis In: Humana Press, editor **Stat. Hum. Genet. Methods Mol. Biol 2017:485–504 1666, New York, NY: Humana Press, New York, NYhttps://doi.org/https://doi.org/10.1007/978-1-4939-7274-6_24.**
35. Solé X, Guinó E, Valls J, et al. SNPStats: A web tool for the analysis of association studies. **Bioinformatics 2006;22:1928–1929 https://doi.org/http://dx.doi.org/10.1093/bioinformatics/btl268.**
36. Smith CL, O'Malley BW. Coregulator function: A key to understanding tissue specificity of selective receptor modulators. **Endocr Rev 2004;25:45–71 https://doi.org/10.1210/er.2003-0023.**
37. Boese AC, Kim SC, Yin K, et al. Sex differences in vascular physiology and pathophysiology : Estrogen and androgen signaling in health and disease. **Am J Physiol Hear Circ Physiol 2017;313: H524–H545 https://doi.org/10.1152/ajpheart.00217.2016.**
38. Swaab DF. Sexual differentiation of the human brain: relevance for gender identity, transsexualism and sexual orientation. **Gynecol Endocrinol 2004;19:301–312.**
39. Yore MA, Im D, Webb LK, et al. Steroid receptor coactivator-2 expression in brain and physical associations with steroid receptors. **Neuroscience 2010;169:1017–1028 https://doi.org/10.1016/j.neuroscience.2010.05.053.**
40. Molenda-Figueira HA, Murphy SD, Shea KL, et al. Steroid receptor coactivator-1 from brain physically interacts differentially with steroid receptor subtypes. **Endocrinology 2008;149:5272–5279 https://doi.org/10.1210/en.2008-0048.**
41. Auger AP, Tetel MJ, McCarthy MM. Steroid receptor coactivator-1 (SRC-1) mediates the development of sex-specific brain morphology and behavior. **Proc Natl Acad Sci 2002;97:7551–7555 https://doi.org/10.1073/pnas.97.13.7551.**