Chapter

An Analysis of the Implication of Estrogens and Steroid Receptor Coactivators in the Genetic Basis of Gender Incongruence

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Abstract

In mammals, sex differences in the adult brain are established very early in development, when the brain is still very immature. 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. Then the estradiol acts by binding to intracellular estrogen receptors (ERs) located predominantly in neurons, masculinizing specific brain regions. But ERs are also transcription factors that, when they are exposed to their ligand, dimerize and form complexes with coactivator proteins and corepressors, modifying the transcription of multiple target genes in a cascade effect and ultimately neuronal function. Given the intimate relationship between steroids and brain dimorphism, and steroid coactivators and gene transcription, in the present work, we further explore the implication of ERs α and β, and steroid coactivators NCoA-1, NCoA-2, NCoA-3, NCoA-4, NCoA-5 and p300-CREBBP, in the genesis of brain dimorphism. Based on our data, we believe that the coactivators NCOA-1, NCOA-2 and p300-CREBBP could be considered as candidate genes for GI.

Keywords: estrogens, gender incongruence, steroid coactivators

1. Introduction

1.1 Gender incongruence

The term *gender identity* refers to "a person's innermost concept of self as male, female, a blend of both or neither, how individuals perceive themselves and what they call themselves" [1, 2], while *sex* refers to the biological sex characteristics based on chromosomal, hormonal, physical, and anatomical characteristics.

Most people present an alignment of gender identity with natal sex (cisgender individuals), but in some cases (transgender individuals) gender identity differs (in varying degrees) from the sex assigned at birth. Thus, Gender Dysphoria (GD) in the Diagnostic and Statistical Manual of Mental Disorders DSM-5 [3] or Gender Incongruence (GI) in the International Classification of Diseases ICD-11 [4] are

characterized by a marked incongruence between one's experienced gender and the sex assigned at birth.

2. The genetic and epigenetic basis of the gender incongruence

The origin of GI is complex and appears to be multifactorial. Current hypotheses point out that GI could be associated with a characteristic neurodevelopmental processes of the brain [5, 6], not concordant with gender, due to the influence of testosterone, converted into estradiol in the brain.

Traditionally, this process of brain masculinization *versus* feminization, has been exclusively analyzed from a hormonal perspective. But in the past two decades, it has been found that this point of view is incomplete, since other important factors such as epigenetics or genetics, for example, are not taken into account.

2.1 The genetic component

A genetic component should also be taken into account since different genes start to express before the formation of the testes [7, 8]. In fact, in mammals, sexual differentiation begins at the time of fertilization, through a different chromosomal complement in males and females, and will be driven by the *SRY* gene, which will guide the undifferentiated gonad towards the formation of the testes. Then, the testosterone will masculinize specific regions of the brain, either directly, or indirectly, through the action of the aromatase [9, 10].

Most studies about the genetic basis of GI analyzed the implication of some DNA polymorphisms related to ERs, α and β, the AR, the aromatase CYP19A1 or the CYP17A1 [11–20] as well as the interaction effects (epistasis) among them [15, 21]. A summary of the principal studies about the genetic component of GI is shown in **Table 1**. This gene selection is based on the hypothesis that a small variation in the DNA sequence of these genes would imply a high variability in the sensitivity of the hormonal receptors to their ligands.

Henningsson et al. [11] were the first group to analyze three repeat polymorphisms, located in the estrogen receptor beta, the androgen receptor, and the aromatase genes in a trans female population. They found a relationship between the number of repetitions and gender incongruence. They found longer estrogen receptor and androgen receptor polymorphisms in the trans female population. Later, Hare et al. [12] replicated Henningsson's study in a bigger population, finding longer androgen receptor polymorphisms. However, when Ujike et al., [13] analyzed the same polymorphisms in a Japanese population, they did not find any statistical difference. These and others polymorphisms were analyzed in a Spanish population by our group. Our results confirmed the involvement of both estrogen receptors (alpha and beta) in gender incongruence. Part of this data was confirmed by Foreman et al. [15].

2.2 The epigenetic component

An epigenetic component may also be involved since there is evidence that some environmental factors play a role in the sexual differentiation of the brain. For example, in mice, the sex difference in maternal anogenital licking of male compared with female pups produces a different methylation of the estrogen receptor α promoter in the preoptic area [22]. And in humans, certain environmental factors, such as a short crossover hormonal treatment (only 6 months), can modify the methylation profile of the estrogen receptor α promoter in a trans population [23, 24].

Investigations	Genes	Populations
Henningsson et al. [11]	ERβ, AR, CYP19A1	29 transwomen
Hare et al. [12]	ERβ, AR, CYP19A1	112 transwomen
Ujikce et al. [13]	ERβ, AR, CYP19A1	168 transmen 74 transwomen
Fernández et al. [14, 16]	ERβ, AR, CYP19A1	273 transmen 442 transwomen
Bentz et al. [19]	CYP17A1	49 transmen 104 transwomen
Fernández et al. [20] (2016)	CYP17A1	223 transmen 317 transwomen
Cortés Cortés et al. [17]	ERα: (TA)n-rs3138774, PvuII-rs2234693, XbaI-rs9340799	183 transmen 184 transwomen
Fernández et al. [21]	ERα, ERβ, AR, CYP19A	425 transmen 549 transwomen
Foreman et al. [15]	AR, ERα, ERβ, SRD5A2, STS, SULT2A1, PGR, COMT, CYP17, SRD5A2	380 transwomen
Fernández et al. [18]	ERα: rs9478245, rs3138774 rs2234693, rs9340799	226 transmen 273 transwomen
Aranda et al. [24]	Epigenetics: ERα, ERβ, AR	12 transmen 6 transwomen
Fernández et al. [23]	Epigenetics: ERα promoter	10 transmen 10 transwomen

Table 1.
Mean investigations about the genetic basis of gender incongruence.

3. Estrogens, androgens and their receptors and coactivators

3.1 Estrogens

Estradiol (E2) exerts a wide variety of effects on growth, development, the function of reproductive systems and regulation in the central nervous system [25, 26]. The mechanism of action of the two ER isoforms α and β consists of binding with the E2 ligand to obtain the receptor's dimerization (αα, αβ or ββ), originating the necessary conformational changes in the ligand binding domain (LBD) [27] and binding with high affinity to specific DNA sequences called estrogen response elements (EREs) [27] in the genes that are regulated by E2 (**Figure 1**). This conformational change in the LBD allows coactivators and other co-regulating proteins to be recruited. We must point out that this step is critical for the transcriptional regulation of genes induced by E2 [28].

Furthermore, estrogens are produced in many regions of the brain including the cortex, the hippocampus, the cerebellum, the hypothalamus and the amygdala, among others [29]. The actions of estrogens in the developing brain are generally permanent and range from the establishment of sexual differences to generalized trophic and neuroprotective effects [30]. In addition, estrogens are an important regulator of brain growth and differentiation, and ERs α and β are found in both the developing [31] and the adult human brain [32].

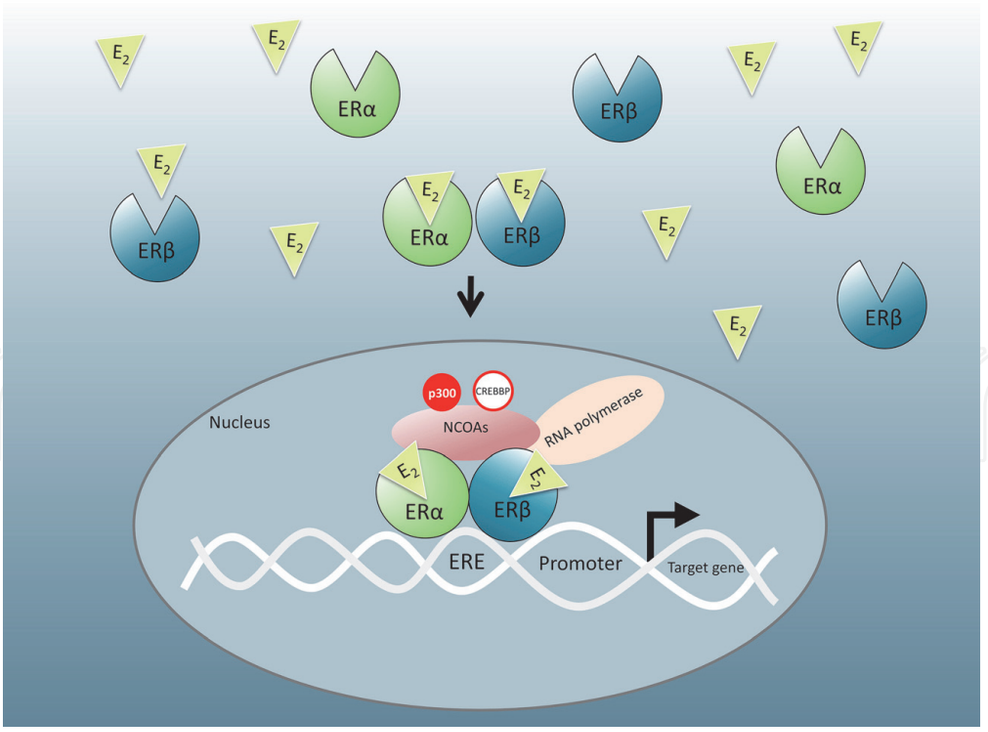

Figure 1.
Molecular mechanisms of action of ERs α and β. hormone 17β-estradiol (E2) binds to the nuclear receptor (ERα or ERβ), and after dimerization and translocation to the nucleus, the nuclear receptor complex binds to a specific sequence of DNA known as an estrogen response element (ERE). The nuclear receptor complex in turn recruits the coactivators NCOAs, p300 and the CREBBP that activate the transcription of target genes.

3.2 Estrogen and androgen receptors are transcription factors

We must point out that the packaging of DNA into chromatin causes a general repression of gene activity, and transcription factors function to relieve this chromatin-mediated repression [33].Thereby, once attached to their ligands, the receptors dimerize, enter the nucleus, and interact with the promoter regions of the target genes, modulating the expression of multiple genes in collaboration with some steroid coactivators (**Figure 1**). In the case of the AR, its ligand is androgen [34] while for the ERs, it is estrogen, 17β-Estradiol (E2) in particular [26].

3.3 Steroid receptor coactivators

Proteins called SRCs (Steroid Receptor Coactivators) serve as primary coactivators that interact with the complex formed by E2 and the hormonal receptor. Additionally, SRCs recruit multiple secondary coactivators such as p300 and the CREB-binding protein (also known as CREBBP) [35]. Both SRCs and p300 are the first coactivators that are coupled to the E2-ER complex [36] to activate the transcriptional process of the genes that are E2 targets (**Figure 1**).

Coactivators are proteins that influence the ability of the transcription factors to activate or inhibit expression of multiple genes in a cascade mode [37]. Given the intimate relationship between steroids and brain dimorphism, and coactivators and gene transcription, and since ERs α-β and the AR are hormonal receptors that act as transcription factors, it was clear that we should hypothesize the implication of DNA coactivators in the process of GI.

4. The role of E2-coactivators in the genetic basis of gender incongruence

To our knowledge, no studies have been published about the role of steroid receptor coactivators in the genetic basis of GI. Nevertheless, given the importance of estrogens in GI, and the critical role of coactivators in the transcriptional gene regulation induced by E2, our team deemed it interesting to analyze 247 single nucleotide polymorphisms (SNPs) located at the coactivators NCoA-1(or SRC-1), NCoA-2 (or SRC-2), NCoA-3 (or SRC-3), NCoA-4, NCoA-5 and p300-CREBBP, in a transgender *versus* a cisgender population, because variation at the DNA level at steroid receptor coactivators could affect the sensitivity of the E2-ER complex, and consequently could modify the transcription of the genes regulated by E2. Some of these data are being published, and the results of the whole study are presented in this chapter.

4.1 The characteristics of the study

Genomic DNA was extracted from 94 Spanish transgender individuals (47 transmen and 47 transwomen) *versus* 94 Spanish cis gender individuals (44 cismen and 50 ciswomen). The transgender population was diagnosed and recruited through the Gender Unit of the Clínic Hospital of Barcelona (Spain) and the cisgender population was selected from a country census (Pizarra) matching by geographic origin and race.

We analyzed 247 polymorphisms distributed in the coactivators NCOA-1 (63 SNPs), NCOA-2 (64 SNPs), NCOA-3 (30 SNPs), NCOA-4 (4 SNPs), NCOA-5 (8 SNPs), p300 (9 SNPs) and CREBBP (69 SNPs) (**Table 2**), in a population of 94 Spanish transgender individuals *versus* 94 Spanish cisgender individuals, with the same geographic origin, race and biological sex. All the polymorphisms were in Hardy–Weinberg equilibrium.

4.2 The findings

4.2.1 Similar prevalence rates for the analyzed polymorphisms and comparison with the global and European 1000 genomes

As expected, the prevalence rates for all analyzed polymorphisms in our population were similar to those found in the Global 1000 genomes and the European 1000 genomes http://www.1000genomes.org (**Table 3**).

4.2.2 Eleven polymorphism showed differences in the distribution of the allele and genotype frequencies

When we compared the distribution of the allele and genotype frequencies, we found significant differences in 11 polymorphisms, that correspond to 4.45% of the total analyzed: three polymorphisms located in NCOA-1, five in NCOA-2, two in p300 and one in CREBBP (**Tables 2** and **3**). The description of the significant association analyses with GI in different models of inheritance is in **Table 4**.

P1 polymorphism: The genotype T/T was overrepresented in the cis population ($P < 0.035$ for dominant model) while the genotypes T/C-C/C were more frequent in the trans population (OR = 2.12*;* $P < 0.038$). The genotype distribution was also significant for the log-additive model (OR = 2.15; $P < 0.027$).

Gene	Chromosome	Function	Analyzed SNPs	SNPs with significant differences
NCOA-1	2	The protein encoded by this gene acts as a transcriptional coactivator for steroid and nuclear hormone receptors.	63	3
NCOA-2	8	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
NCOA-3	20	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
NCOA-4	10	This gene encodes an androgen receptor coactivator.	4	0
NCOA-5	20	This gene encodes a coregulator for the α and β estrogen receptors.	8	0
p300	22	This gene encodes a transcriptional coactivator protein.	9	2
CREBBP	16	This gene is ubiquitously expressed and is involved in the transcriptional coactivation factors.	69	1
		TOTAL	**247**	**11**
		%	**100**	**4.45**

Table 2.
Description of the analyzed polymorphisms.

Gene	Polymorphism	Alias	DNA variation	Our study frequency	Global 1000 genomes frequency	European 1000 genomes frequency
NCOA-1	rs10495747	P1	T/C	C = 0.11	C = 0.1330	C = 0.1153
	rs2584940	P2	T/G	G = 0.38	G = 0.4605	G = 0.4125
	rs6756785	P3	A/G	G = 0.32	G = 0.2115	G = 0.2883
NCOA-2	rs76968380	P4	G/A	A = 0.06	A = 0.1138	A = 0.0646
	rs34406737	P5	G/A	A = 0.15	A = 0.1300	A = 0.1262
	rs1963250	P6	G/T	T = 0.57	T = 0.5691	T = 0.5368
	rs10755950	P7	G/A	A = 0.42	A = 0.5655	A = 0.4483
	rs56055423	P8	A/G	G = 0.05	G = 0.0132	G = 0.0457
p300	rs133084	P9	T/C	C = 0.5956	C = 0.5447	C = 0.59
	rs11806	P10	A/G	G = 0.3894	G = 0.3499	G = 0.42
CREBBP	rs2191416	P11	G/A	A = 0.2660	A = 0.2555	A = 0.26

Table 3.
Description of the polymorphisms with significant differences.

P2 polymorphism: The genotype T/T was overrepresented in the cis population ($P < 0.044$), while the genotypes T/G (OR = 1.97; $P < 0.035$) and G/G (OR = 2.65; $P < 0.045$) were overrepresented in the trans population (codominant model). The

genotype distribution for P2 was also significant for the dominant and log-additive models.

P3 polymorphism: The genotype A/A was overrepresented in the cis population ($P < 0.0079$ for the dominant model), while the genotypes A/G-G/G were overrepresented in the trans population (OR = 2.20; $P < 0.009$). The genotype distribution for P3 was significant for the codominant, dominant, overdominant and log-additive models.

P4 polymorphism: The P4 polymorphism was only significant for the log-additive model. The genotype G/G was overrepresented in the cis population, while the genotypes G/A and A/A were overrepresented in the trans population (OR = 2.57; $P < 0.034$ for the log-additive model).

P5 polymorphism: This polymorphism was significant for the codominant, the recessive, and the overdominant models. The G/G and the A/A genotypes were overrepresented in the trans population ($P < 0.0029$; codominant model)) while the G/A was overrepresented in the cis population (OR = 0.48; $P < 0.036$).

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; $P < 0.008$ for the dominant model). The genotype distribution was significant for the codominant, dominant and log-additive models.

P7 polymorphism: This polymorphism was only significant for the recessive model. The A/A genotype was overrepresented in the trans population (OR = 2.44; $P < 0.026$, recessive model).

P8 polymorphism: The genotype A/A was overrepresented in the cis population ($P < 0.0068$ dominant model) while the genotype A/G was overrepresented in the trans population (OR = 4.49; $P < 0.024$, codominant model). This polymorphism showed significant differences for the codominant, dominant, overdominant, and log-additive models. Only the recessive model did not show significant results.

P9 polymorphism: The C/C genotype was overrepresented in the trans population, while the T/C and T/T were overrepresented in the cis population (OR = 0.50; $P < 0.025$, dominant model).

P10 polymorphism: This polymorphism was only significant for the log-additive model. The G/G genotype was overrepresented in the trans population (OR = 2.69; $P < 0.030$, codominant model).

P11 polymorphism: The A/A polymorphism was overrepresented in the trans population (OR = 4.82; $P < 0.048$, recessive model), while G/G-A/G were more frequent in the cis population ($P < 0.025$).

4.2.3 The three polymorphisms (P2, P9 and P10) showed significant differences in the interaction analysis with the covariate "sex"

Furthermore, polymorphisms P2, P9 and P10 showed significant differences in the interaction analysis with covariate "sex". For the P2 polymorphism, the genotype T/G was more frequent in the trans population assigned as females at birth than in the cis female population (OR = 2.76; $P < 0.029$) while the genotype G/G was more frequent in the trans population assigned as males than in the cis male population (OR = 8.0; $P < 0.016$).

While for the P9 polymorphism, the genotype T/T was more frequent in the cis female population than in the trans population assigned as females at birth (OR = 0.34; $P < 0.014$). And finally, the genotypes A/G-G/G for the P10 polymorphism were more frequent in the trans population assigned as females at birth than in the cis female population (OR = 2.68; $P < 0.031$).

Model	Genotype	Cis (%)	Trans (%)	OR	*P*-value
P1 polymorphism (rs10495747)					
Codominant	T/T	79 (84%)	67 (71.3%)	1.00	0.068
	T/C	15 (16%)	26 (27.7%)	2.04 (1.00–4.17)	**0.05***
	C/C	0 (0%)	1 (1.1%)	NA (0.00-NA)	—
Dominant	T/T	79 (84%)	67 (71.3%)	1.00	**0.035***
	T/C-C/C	15 (16%)	27 (28.7%)	2.12 (1.04–4.32)	**0.038***
Recessive	T/T–T/C	94 (100%)	93 (98.9%)	1.00	0.24
	C/C	0 (0%)	1 (1.1%)	NA (0.00-NA)	—
Overdominant	T/T-C/C	79 (84%)	68 (72.3%)	1.00	0.051
	T/C	15 (16%)	26 (27.7%)	2.01 (0.99–4.11)	0,054
Log-additive	—	—	—	2.15 (1.07–4.29)	**0.027***
P2 polymorphism (rs2584940)					
Codominant	T/T	43 (45.7%)	27 (28.7%)	1.00	**0.044***
	T/G	42 (44.7%)	52 (55.3%)	1.97 (1.05–3.70)	**0.035***
	G/G	9 (9.6%)	15 (16%)	2.65 (1.02–6.91)	**0.045***
Dominant	T/T	43 (45.7%)	27 (28.7%)	1.00	**0.015***
	T/G-G/G	51 (54.3%)	67 (71.3%)	2.09 (1.14–3.83)	**0.017***
Recessive	T/T–T/G	85 (90.4%)	79 (84%)	1.00	0.19
	G/G	9 (9.6%)	15 (16%)	1.79 (0.74–4.33)	0.198
Overdominant	T/T-G/G	52 (55.3%)	42 (44.7%)	1.00	0.14
	T/G	42 (44.7%)	52 (55.3%)	1.53 (0.86–2.72)	0.148
Log-additive	—	—	—	1.72 (1.10–2.69)	**0.015***
P3 polymorphism (rs6756785)					
Codominant	A/A	50 (53.2%)	32 (34%)	1.00	**0.029***
	A/G	38 (40.4%)	54 (57.5%)	2.22 (1.21–4.08)	**0.010***
	G/G	6 (6.4%)	8 (8.5%)	2.08 (0.66–6.56)	0.213
Dominant	A/A	50 (53.2%)	32 (34%)	1.00	**0.0079***
	A/G-G/G	44 (46.8%)	62 (66%)	2.20 (1.22–3.97)	**0.009***
Recessive	A/A-A/G	88 (93.6%)	86 (91.5%)	1.00	0.58
	G/G	6 (6.4%)	8 (8.5%)	1.36 (0.45–4.10)	0.598
Overdominant	A/A-G/G	56 (59.6%)	40 (42.5%)	1.00	**0.019***
	A/G	38 (40.4%)	54 (57.5%)	1.99 (1.11–3.55)	**0.020***
Log-additive	—	—	—	1.77 (1.10–2.87)	**0.017***
P4 polymorphism (rs76968380)					
Codominant	G/G	88 (93.6%)	80 (85.1%)	1.00	0.075
	G/A	6 (6.4%)	12 (12.8%)	2.20 (0.79–6.14)	0.132
	A/A	0 (0%)	2 (2.1%)	NA (0.00-NA)	—
Dominant	G/G	88 (93.6%)	80 (85.1%)	1.00	0.055
	G/A-A/A	6 (6.4%)	14 (14.9%)	2.57 (0.94–7.00)	0.065

Model	Genotype	Cis (%)	Trans (%)	OR	*P*-value
Recessive	G/G-G/A	94 (100%)	92 (97.9%)	1.00	0.095
	A/A	0 (0%)	2 (2.1%)	NA (0.00-NA)	—
Overdominant	G/G-A/A	88 (93.6%)	82 (87.2%)	1.00	0.13
	G/A	6 (6.4%)	12 (12.8%)	2.15 (0.77–5.98)	0.144
Log-additive	—	—	—	2.57 (1.01–6.55)	**0.034***
P5 polymorphism (rs34406737)					
Codominant	G/G	63 (67%)	72 (76.6%)	1.00	**0.0029***
	G/A	31 (33%)	17 (18.1%)	0.48 (0.24–0.95)	**0.036***
	A/A	0 (0%)	5 (5.3%)	NA (0.00-NA)	—
Dominant	G/G	63 (67%)	72 (76.6%)	1.00	0.14
	G/A-A/A	31 (33%)	22 (23.4%)	0.62 (0.33–1.18)	0.142
Recessive	G/G-G/A	94 (100%)	89 (94.7%)	1.00	**0.0078***
	A/A	0 (0%)	5 (5.3%)	NA (0.00-NA)	—
Overdominant	G/G-A/A	63 (67%)	77 (81.9%)	1.00	**0.018***
	G/A	31 (33%)	17 (18.1%)	0.45 (0.23–0.88)	**0.020***
Log-additive	—	—	—	0.85 (0.49–1.49)	0.57
P6 polymorphism (rs1963250)					
Codominant	T/T	20 (21.3%)	37 (39.4%)	1.00	**0.015***
	T/G	54 (57.5%)	46 (48.9%)	0.46 (0.24–0.90)	**0.021***
	G/G	20 (21.3%)	11 (11.7%)	0.30 (0.12–0.74)	**0.009***
Dominant	T/T	20 (21.3%)	37 (39.4%)	1.00	**0.0067***
	T/G-G/G	74 (78.7%)	57 (60.6%)	0.42 (0.22–0.79)	**0.008***
Recessive	T/T–T/G	74 (78.7%)	83 (88.3%)	1.00	0.075
	G/G	20 (21.3%)	11 (11.7%)	0.49 (0.22–1.09)	0.080
Overdominant	T/T-G/G	40 (42.5%)	48 (51.1%)	1.00	0.24
	T/G	54 (57.5%)	46 (48.9%)	0.71 (0.40–1.26)	0.245
Log-additive	—	—	—	0.53 (0.34–0.83)	**0.0043***
P7 polymorphism (rs10755950)					
Codominant	G/G	33 (35.1%)	31 (33%)	1.00	0.064
	A/G	50 (53.2%)	40 (42.5%)	0.85 (0.45–1.62)	0.632
	A/A	11 (11.7%)	23 (24.5%)	2.23 (0.93–5.31)	0.071
Dominant	G/G	33 (35.1%)	31 (33%)	1.00	0.76
	A/G-A/A	61 (64.9%)	63 (67%)	1.10 (0.60–2.01)	0.770
Recessive	G/G-A/G	83 (88.3%)	71 (75.5%)	1.00	**0.022***
	A/A	11 (11.7%)	23 (24.5%)	2.44 (1.11–5.36)	**0.026***
Overdominant	G/G-A/A	44 (46.8%)	54 (57.5%)	1.00	0.14
	A/G	50 (53.2%)	40 (42.5%)	0.65 (0.37–1.16)	0.14
Log-additive	—	—	—	1.35 (0.90–2.04)	0.15

Model	Genotype	Cis (%)	Trans (%)	OR	*P*-value
P8 polymorphism (rs56055423)					
Codominant	A/A	91 (96.8%)	81 (86.2%)	1.00	**0.021***
	A/G	3 (3.2%)	12 (12.8%)	4.49 (1.22–16.49)	**0.024***
	G/G	0 (0%)	1 (1.1%)	NA (0.00-NA)	—
Dominant	A/A	91 (96.8%)	81 (86.2%)	1.00	**0.0068***
	A/G-G/G	3 (3.2%)	13 (13.8%)	4.87 (1.34–17.69)	**0.016***
Recessive	A/A-A/G	94 (100%)	93 (98.9%)	1.00	0.24
	G/G	0 (0%)	1 (1.1%)	NA (0.00-NA)	—
Overdominant	A/A-G/G	91 (96.8%)	82 (87.2%)	1.00	**0.012***
	A/G	3 (3.2%)	12 (12.8%)	4.44 (1.21–16.29)	**0.024***
Log-additive	—	—	—	4.69 (1.32–16.63)	**0.0057***
P9 polymorphism (rs133084)					
Codominant	C/C	27 (28.7%)	42 (44.7%)	1.00	0.056
	T/C	46 (48.9%)	39 (41.5%)	0.55 (0.29–1.04)	0.066
	T/T	21 (22.3%)	13 (13.8%)	0.40 (0.17–0.93)	**0.034***
Dominant	C/C	27 (28.7%)	42 (44.7%)	1.00	**0.023***
	T/C-T/T	67 (71.3%)	52 (55.3%)	0.50 (0.27–0.91)	**0.025***
Recessive	C/C-T/C	73 (77.7%)	81 (86.2%)	1.00	0.13
	T/T	21 (22.3%)	13 (13.8%)	0.56 (0.26–1.19)	0.135
Overdominant	C/C-T/T	48 (51.1%)	55 (58.5%)	1.00	0.3
	T/C	46 (48.9%)	39 (41.5%)	0.74 (0.42–1.32)	0.307
Log-additive	—	—	—	0.61 (0.41–0.93)	**0.019***
P10 polymorphism (rs11806)					
Codominant	A/A	37 (39.8%)	25 (26.6%)	1.00	0.076
	A/G	45 (48.4%)	49 (52.1%)	1.61 (0.84–3.08)	0.151
	G/G	11 (11.8%)	20 (21.3%)	2.69 (1.10–6.58)	**0.030***
Dominant	A/A	37 (39.8%)	25 (26.6%)	1.00	0.055
	A/G-G/G	56 (60.2%)	69 (73.4%)	1.82 (0.98–3.38)	0.058
Recessive	A/A-A/G	82 (88.2%)	74 (78.7%)	1.00	0.08
	G/G	11 (11.8%)	20 (21.3%)	2.01 (0.91–4.48)	0.086
Overdominant	A/A-G/G	48 (51.6%)	45 (47.9%)	1.00	0.61
	A/G	45 (48.4%)	49 (52.1%)	1.16 (0.65–2.06)	0.627
Log-additive	—	—	—	1.63 (1.06–2.52)	**0.023***
P11 polymorphism (rs2191416)					
Codominant	G/G	53 (57%)	47 (50%)	1.00	0.075
	A/G	38 (40.9%)	38 (40.4%)	1.13 (0.62–2.05)	0.702
	A/A	2 (2.1%)	9 (9.6%)	5.07 (1.04–24.67)	**0.044***
Dominant	G/G	53 (57%)	47 (50%)	1.00	0.34
	A/G-A/A	40 (43%)	47 (50%)	1.32 (0.74–2.36)	0.354
Recessive	G/G-A/G	91 (97.8%)	85 (90.4%)	1.00	**0.025***
	A/A	2 (2.1%)	9 (9.6%)	4.82 (1.01–22.93)	**0.048***

Model	Genotype	Cis (%)	Trans (%)	OR	*P*-value
Overdominant	G/G-A/A	55 (59.1%)	56 (59.6%)	1.00	0.95
	A/G	38 (40.9%)	38 (40.4%)	0.98 (0.55–1.76)	0.951
Log-additive	—	—	—	1.49 (0.92–2.41)	0.1

Table 4.
Polymorphism association analysis with gender incongruence, in different models of inheritance (Codominant, Dominant, Recessive, Overdominant and Log-additive) (n = 188, crude analysis).

4.2.4 The haplotype analysis of the coactivators NCOA-1, NCOA-2 and p300, and comparison between cis and trans population

The simultaneous analysis of multiple loci (haplotypes) was carried out in those coactivators with two or more polymorphisms with statistical significance (NCOA-1, NCOA-2 and p300) using logistic regression models.

4.2.4.1 Polymorphisms in NCOA-1

For the three polymorphisms located in NCOA-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. This haplotype was more frequent in the cis than in the trans population. The haplotype 5: C-G-A was overrepresented in the trans population and showed statistical significance (OR = 2.62; $P < 0.05$). The P global haplotype association was $P < 0.009$.

4.2.4.2 Polymorphisms in NCOA-2

For the five polymorphisms located in NCOA-2 (**Table 6**), the significant haplotypes were the haplotype 2: (G-G-T-A-A) (OR = 2.49; $P < 0.02$) and the haplotype 8: (G-G-T-A-G) (OR = 12.86; $P < 0.028$), with a P global haplotype association $P < 0.005$. Both polymorphisms were overrepresented in the trans population.

4.2.4.3 Polymorphisms in p300

For the two polymorphisms located in p300 (**Table 7**), the significant haplotype was haplotype 2 (T-A) (OR = 0.57; $P < 0.018$) with a P global haplotype association $P < 0.033$. This haplotype was more frequent in the cis than in the trans population, and it was only significant in the population with a female natal sex (biological sex) (OR = 0.43; $P < 0.013$) (**Table 8**).

4.2.4.4 Summary of findings

In summary, when we analyzed the allele and genotype frequencies, we found significant differences in 11 polymorphisms located in NCOA-1, NCOA-2, p300 and CREBBP. Being the NCOA-2 and p300 the coactivators with the highest percentages of polymorphisms with significant differences (5/64 and 2/9 respectively). Furthermore, only P2 (located at NCOA-1), P9 (located at p300) and P10 (located at p300) showed a different distribution of the genotypes in males and females, that is, they showed significant differences in the interaction analysis with covariate "sex".

Regarding the haplotype analysis, there were four polymorphisms with significant differences: the haplotype 5 (C-G-A) in NCOA-1, the haplotype 2

Haplotype frequencies estimation and haplotype association with GI (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	—
2	T	T	G	0.1495	0.134	0.1699	0.5996	2.25 (0.99–5.13)	0.054
3	T	G	G	0.147	0.1319	0.1601	0.7466	1.73 (0.81–3.71)	0.16
4	T	G	A	0.139	0.1341	0.144	0.8856	1.80 (0.81–3.97)	0.15
5	C	G	A	0.069	0.0531	0.0897	0.9546	2.62 (1.00–6.83)	**0.05***
6	C	T	A	0.0228	0.0267	0.0169	0.9774	1.39 (0.23–8.34)	0.72
7	C	G	G	0.0226	0	0.0423	1	379142884.10 (379142883.16–379142885.04)	**<0.0001***

**Global haplotype association P-value: 0.009.*

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

Haplotype frequencies estimation and haplotype association with GI (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	—
2	G	G	T	A	A	0.2206	0.2142	0.236	0.4752	2.49 (1.16–5.34)	**0.02***
3	G	G	T	G	A	0.2022	0.1721	0.2303	0.6773	2.00 (0.93–4.31)	0.079
4	G	G	G	A	A	0.0891	0.0861	0.0793	0.7664	1.05 (0.38–2.88)	0.92
5	G	A	G	G	A	0.0474	0.0656	0.0235	0.8138	0.55 (0.11–2.89)	0.48
6	G	A	T	A	A	0.0419	0.0474	0.0423	0.8557	1.11 (0.24–5.24)	0.89
7	G	A	T	G	A	0.041	0.0393	0.0426	0.8967	3.00 (0.10–86.09)	0.52
8	G	G	T	A	G	0.0217	0	0.036	0.9184	12.86 (1.34–123.38)	**0.028***
9	A	G	T	G	A	0.0173	0.0085	0.0228	0.9357	5.62 (0.56–56.71)	0.15
10	A	G	G	G	A	0.0165	0.0077	0.0205	0.9522	3.53 (0.40–31.50)	0.26
11	G	A	T	A	G	0.0138	0.0028	0.0189	0.9659	2.68 (0.27–26.20)	0.4
12	A	G	T	A	A	0.0108	0.0158	0.0093	0.9767	0.00 (-Inf - Inf)	1
rare	*	*	*	*	*				1	2688767424666564139418529253819445824958476503371363684397875 2.00 (2688767424662284679936719703712080660307679137154433656920 4736.00–2688767424670843598900338803926810989609273869588293711 8752.00)	**<0.0001***

Global haplotype association P-value: 0.005.

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

Haplotype frequencies estimation and Haplotype association with GI (n = 188, adjusted by sex)								
Haplotypes	**P9**	**P10**	**Total**	**Cis population**	**Trans population**	**Cumulative frequency**	**OR (95% CI)**	***P*-value**
1	C	G	0.4103	0.3468	0.4734	0.4103	1.00	—
2	T	A	0.4004	0.4549	0.3457	0.8107	0.57 (0.36–0.90)	**0.018***
3	C	A	0.1828	0.1851	0.1809	0.9935	0.69 (0.38–1.27)	0.24
4	T	G	0.0065	0.0132	0	1	0.00 (-Inf - Inf)	1

**Global haplotype association P-value: 0.033.*

Table 7.
Haplotype analysis for polymorphisms located in p300 (P9 and P10 polymorphisms).

Haplotype and sex cross-classification interaction table (n = 188, crude analysis)					
		Females		**Males**	
Haplotype	**Frequency**	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**
1	0.4102	1.00	—	0.63 (0.19–2.07)	0.457
2	0.4003	0.43 (0.22–0.83)	**0.013***	0.49 (0.19–1.27)	0.141
3	0.1829	0.60 (0.26–1.40)	0.237	0.52 (0.18–1.52)	0.232
Rare	0.0066	0.00 (0.00 - Inf)	—	0.00 (-Inf - Inf)	—
Haplotypes within sex (n = 188, crude analysis)					
		Females		**Males**	
Haplotype	**Frequency**	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**
1	0.4102	1.00	—	1.00	—
2	0.4003	0.43 (0.22–0.83)	**0.013***	0.77 (0.40–1.49)	0.444
3	0.1829	0.60 (0.26–1.40)	0.237	0.82 (0.35–1.96)	0.665
Rare	0.0066	0.00 (0.00 - Inf)	—	0.00 (0.00 - Inf)	—
Sex within haplotypes (n = 188, crude analysis)					
		Females	**Males**		
Haplotype	**Frequency**	**OR (95% CI)**	**OR (95% CI)**	***P*-value**	
1	0.4102	1.00	0.63 (0.19–2.07)	0.457	
2	0.4003	1.00	1.12 (0.55–2.30)	0.769	
3	0.1829	1.00	0.87 (0.32–2.34)	0.796	
Rare	0.0066	1.00	Inf		

Table 8.
Haplotype interaction analysis with covariate sex for polymorphisms located in p300 (P9 and P10 polymorphisms). Haplotype frequency, Odds ratio (OR) and P-value in female and male populations.

(G-G-T-A-A) in NCOA-2, the haplotype 8 (G-G-T-A-G) also in NCOA-2, and the haplotype 2 (T-A) in p300. These NCOA-1 and NCOA-2 significant haplotypes were more frequent in the trans population (OR = 2.62, OR = 2.49 and OR = 12.86, respectively) while the haplotype 2 (T-A) in p300 was more frequent in the cis population (OR = 0.57). The NCOA-2 haplotype 8 (OR = 12.86; $P < 0.028$) had a strikingly much higher value than the others. That is due to the fact that this haplotype only occurred in the trans population.

4.3 Concordance of our findings with the literature about receptor coactivators

To our knowledge, no studies have been published about the role of steroid receptor coactivators in the genetic basis of GI. Our data are in concordance with a recent work that showed that the nuclear receptor coactivators, NCOA-1, NCOA-2 and p300, are essential for efficient ER transcriptional activity in the brain [33, 38]. Furthermore, NCOA-1 and NCOA-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, difference in the coupling with the ERs [38, 39]. These differential interactions between NCOA-1 and NCOA-2 with the ER subtypes α and β suggest that these brain regions have distinct expression pattern of co-regulators, and understanding how nuclear receptor coactivators function with various steroid receptors is critical to understanding how 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 NCOA-1 in the developing brain [40]. The authors, Auger et al., investigated the consequence of reducing NCOA-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 NCOA-1 expression is critically involved in the hormone-dependent development of normal male reproductive behavior and brain morphology. Thus, our data are in agreement with the results of Auger et al., [40] since the polymorphic analysis of this coactivator showed significant differences when allelic and genotypic frequencies and haplotypes analyses were carried out.

Our data are also in concordance with other studies about the critical role of p300 and CREBBP in ERα transcription. p300 and CREBBP are two of multiple secondary coactivators recruited by NCOA1, NCOA2 or NCOA3 to form a receptor-coactivator complex that can promote chromatin remodeling and facilitate transcriptional activation [35]. In our work, we found statistical significances in p300, CREBBP, NCOA1 and NCOA2, but not in NCOA3.

Transcription by RNA polymerase II requires the coordinated action of multiple factors such as DNA-binding factors, coactivators, chromatin remodeling, with the basal transcriptional machinery. Futhermore, p300 and CREBBP, do not bind DNA on their own, but they play an essential role in the transcription process mediated by E2 [33, 41]. Thus, ERα functions cooperatively with p300 and CREBBP to increase transcription [42]. Yi et al. [35] demonstrated the quaternary structure of an active complex of DNA-bound ERα, steroid receptor coactivator, and p300 as secondary coactivator. The structural model suggests that the ER binds the ERE-DNA as a dimer and then recruits two NCOAs; these two coactivators, in turn, secure one molecule of p300 to the complex through multiple contacts.

It is very important to maintain the nucleotide sequence of the genes encoding the coactivators in order to maintain the interactions of the ER-E2 -NCOA -p300-CREBBP complex and thus perform the genomic function of estrogens. Therefore, our data are in concordance because finding significant polymorphisms in the sample analyzed may result in ineffective or low effective interactions affecting the E2 target genes involved in brain dimorphism.

In our work, we found 2/9 polymorphisms with statistically significant differences (P9 and P10) in p300 in the interaction analysis with covariate "sex". This implied differences in haplotype distribution according to sex, and thus, the haplotype 2 (T-A) (**Table 8**) only showed significant differences in the population assigned as females at birth. The other haplotypes did not show differences in the distribution between cis and trans population, nor in males or females.

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 [43]. However, the analysis of the androgen coactivator NCOA-4 did not show any significant data.

Currently, it is still very difficult to interconnect molecular, brain, and behavioral findings [44] due to the complex interactions among behavior, genes, hormones, receptors and enzymes. But we must point out that MRI studies in people with GI, show characteristic brain profiles [5]. Both trans populations (females and males) share some common features: firstly, the involvement of the two ERs in neurobiological origin [21] and, secondly, their cortex, in some regions, is thicker than in cismen [5]. These observations support the hypothesis that transmen and transwomen undergo an atypical developmental process with respect to the sexual differentiation of their cortex [5], hypothetically, under the influence of brain estrogens, androgens, their receptors and some of their coactivators.

4.4 Consistency of the findings with the current hypothesis of a multiplicity of mechanisms involved in the complex "mosaic" model of the mammalian brain

Finally, our data are also consistent with the current hypothesis about the existence of a complex "mosaic" model of the mammalian brain [45], with a multiplicity of mechanisms involved, allowing a variable degree of masculinization/feminization within the brain. The simple model according to which testosterone masculinizes the brain of men away from a predetermined female profile, has been replaced by a complex model, according to which sexual effects on the brains of women and men are exerted by a complex combination of behavior, genetic, epigenetic and hormonal factors [45].

5. Conclusions

Based on the data presented here, we believe that it can be stated that there is a genetic basis for GI. Thus, the coactivators, NCOA-1, NCOA-2 and p300-CREBBP could be considered as candidates for increasing the list of potential "susceptibility" genes for GI. Furthermore, our data continue to support the hypothesis that GI is a multifactorial complex trait, involving intricate interactions among genes, steroids, steroids receptors and coactivators.

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Conflict of interest

The authors declare no conflict of interest.

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