SEXUAL MEDICINE ORIGINAL RESEARCH

Analysis of Four Polymorphisms Located at the Promoter of the Estrogen Receptor Alpha *ESR1* Gene in a Population With Gender Incongruence

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ABSTRACT

Introduction: Gender incongruence defines a state in which individuals feel discrepancy between the sex assigned at birth and their gender. Some of these people make a social transition from male to female (trans women) or from female to male (trans men). By contrast, the word cisgender describes a person whose gender identity is consistent with their sex assigned at birth.

Aim: To analyze the implication of the estrogen receptor α gene (*ESR1*) in the genetic basis of gender incongruence.

Main Outcome Measures: Polymorphisms rs9478245, rs3138774, rs2234693, rs9340799.

Method: We carried out the analysis of 4 polymorphisms located at the promoter of the *ESR1* gene (C1 = rs9478245, C2 = rs3138774, C3 = rs2234693, and C4 = rs9340799) in a population of 273 trans women, 226 trans men, and 537 cis gender controls. For SNP polymorphisms, the allele and genotype frequencies were analyzed by χ^2 test. The strength of the SNP associations with gender incongruence was measured by binary logistic regression. For the STR polymorphism, the mean number of repeats were analyzed by the Mann−Whitney *U* test. Measurement of linkage disequilibrium and haplotype frequencies were also performed.

Results: The C2 median repeats were shorter in the trans men population. Genotypes S/S and S/L for the C2 polymorphism were overrepresented in the trans men group ($P = .012$ and $P = .003$ respectively). We also found overtransmission of the A/A genotype (C4) in the trans men population ($P = .017$), while the A/G genotype (C4) was subrepresented ($P = .009$]. The analyzed polymorphisms were in linkage disequilibrium. In the trans men population, the T(C1)-L(C2)-C(C3)-A(C4) haplotype was overrepresented ($P = .019$) while the T(C1)-L(C2)-C(C3)-G(C4) was subrepresented ($P = .005$).

Conclusion: The *ESR1* is associated with gender incongruence in the trans men population. **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;XX:XXX−XXX.**

Key Words: Gender incongruence; transgender; rs9478245; rs3138774; rs2234693; rs9340799

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INTRODUCTION

Gender identities are classified into "transgender" and "cisgender" umbrellas.[1] Transgender is an adjective used to describe a diverse group of individuals whose gender identity is different (in varying degrees) from their sex assigned at birth. Cisgender is used to refer to a gender identity that matches a person's sex assigned at birth.

When an individual applies for transgender health care, he or she may receive a diagnosis based on fulfillment of DSM (Diagnostic and Statistical Manual of Mental Disorders) or ICD (International Classification of Diseases) criteria. Gender incongruence[2] or gender dysphoria[3] criteria defines a condition in which individuals experience a discrepancy between the sex assigned at birth and the gender they identify with. Both terms are more dimensional (less binary) than the previous versions, and therefore, the diagnosis is applicable to a larger group of individuals. Moreover, gender incongruence has been removed from the chapter of Mental and Behavioral Disorders and moved to a new section (Conditions Related to Sexual and Gender Health). Nevertheless, the individuals diagnosed with gender incongruence represent a subsample of the total spectrum of transgender people.[4]

Genetic studies on transgender populations are often based on individuals whose "experienced gender" is more binary, that is, their identification is with "the opposite sex," "preferred sex," or "the other sex" (terms currently used in the ICD-10[5] and DSM-4-TR[6]). Many of these people make a social transition from male to female (trans women) or from female to male (trans men), through cross-sex hormonal treatment and subsequent physical adaptation through surgery.[7]

The origin of gender incongruence seems to be multifactorial. It might be associated with neurodevelopmental processes of the brain.[8–10] A genetic component may also be involved because siblings of transgender individuals are more likely to be transgender, compared with the general population.[11,12] Most genetic studies that analyze the genetic component of gender formation examine the implication of genetic polymorphisms related to the androgen receptor AR,[13–18] the aromatase CYP19A1,[13–18] the estrogen receptors ER (α and β),[13–19] as well as haplotypes and the interaction effects (epistasis) between polymorphisms.[20]

Estrogen is produced in many regions of the brain including the hippocampus, the cortex, the cerebellum, the hypothalamus, and the amygdala.[21] The actions of estradiol in the developing brain are generally permanent and range from the establishment of sexual differences (cerebral dimorphism) to generalized trophic and neuroprotective effects.[22] For this reason, given the importance of estrogens in brain dimorphism, and based on our previous genetic studies,[16,18–20,23,24] we analyzed 4 polymorphisms at/or flanking the promoter of the estrogen receptor alpha *ESR1* gene (Table 1), that encodes the estrogen receptor alpha (ERα), an estrogen-activated transcription factor with a 4-fold higher affinity for estrogens than ER beta (ERβ).[25]

METHODS AND MATERIALS

Participants

The initial population was 323 trans women, 235 trans men, and 252 cis women and 285 cis men controls, recruited through the Gender Units of the Clínic Hospital of Barcelona (Spain) and the Regional Universitario Hospital of Málaga (previously Carlos Haya Hospital) (Spain) since 2010.

To obtain a homogeneous sample, avoiding stratification, we applied the following criteria: The inclusion criteria for the transgender groups were as follows: (1) presenting gender incongruence according to the ICD-11, (2) gender identification with the other gender (male or female), (3) presenting early-life onset of gender nonconformity before or at puberty, (4) being sexually attracted to persons with the same biological sex, (5) having no sexual development disorder, (6) being aged between 18 and 47 years old at enrollment.

At the time of recruitment, transgender people met diagnostic criteria according to ICD-10[5] and DSM-4-TR[6] as assessed by means of several interviews by experienced psychiatrists and psychologists of both teams. To avoid stigmatization of mental disorders, diagnosis was relabeled to gender incongruence.[2] In the current sample, changes do not interfere with the interpretation of our results because transgender participants explicitly reported feelings of belonging to the other gender and communicated the desire for hormonal and surgical gender affirmation treatment.

Early-life onset was established by interviews evaluating whether the gender incongruence was present in childhood (before puberty). Sexual orientation in trans women and trans men was recognized 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. Trans women and trans men underwent endocrinological hormonal assessment to rule out hormonal and sexual development disorders. Clinical and sociodemographic characteristics from a similar transsexual Spanish population attended to at the same reference unit have been described in detail elsewhere.[26]

The DNA samples of the cisgender group were recruited from the biobanc generated for the Pizarra study,[27] in which all participants signed the informed consent for donation of the samples to the biobanc of the Regional Universitario Hospital of Málaga for medical research studies. In the Pizarra study, individuals were excluded if they had been hospitalized for any reason in the 4 weeks before the evaluation, if they were pregnant, or had a severe medical or psychiatric disorder. In addition, cisgender participants were asked about any existing acute or chronic medical conditions, and if they identified themselves as woman, man, or other nonbinary identities. Our inclusion criteria for cis gender subjects were as follows: (1) age between 18 and 47 years old at enrollment; (2) identification as male or female.

Table 1. Description of polymorphisms and PCR conditions to amplify the polymorphic regions

Polymorphism	Alias	Gene	Chromosome	Regulation by Ensembl	Polymorphism type	DNA variation	Digestion enzyme	Global 1000 Genomes frequency	European 1000 Genomes frequency	Our study frequency	Genotypes resulting	Primers
rs9478245	C1	*ESR1*	6q25.1 -q25.2	Promoter 5 prime UTR variant	SNP	T/C	*BsrDI*	C = 0.12	C = 0.02	C = 0.02	C/C C/T T/T	5′-TTCTCCTTTCCCCTGTTCC-3′ 5′-AGACCCCCAGTATTGCAG-TG-3′
rs3138774	C2	″	″	Promoter	STR	(TA)n	–	–	–	–	S/S S/L L/L	5-GACGCATGATATACTTCACC-3′FAM 5-GCAGAATCAAATATCCA-GATG-3′
rs2234693	C3	″	″	Intron promoter_ flanking_ region	SNP	T/C	*PvuII*	C = 0.45	C = 0.42	C = 0.43	C/C C/T T/T	5′-GATATCCAGGGTTATGTGGCA-3′ 5′-AGGTGTTGCCTATTAT-ATTAACCTTGA-3′
rs9340799	C4	″	″	Intron promoter_ flanking_ region	SNP	A/G	*XbaI*	G = 0.28	G = 0.31	G = 0.35	G/G A/G A/A	5′-GATATCCAGGGTTATG-TGGCA-3′ 5′-AGGTGTTGCCTATTATATT-AACCTTGA-3′

SNP = single-nucleotide polymorphism; STR = short tandem repeat.

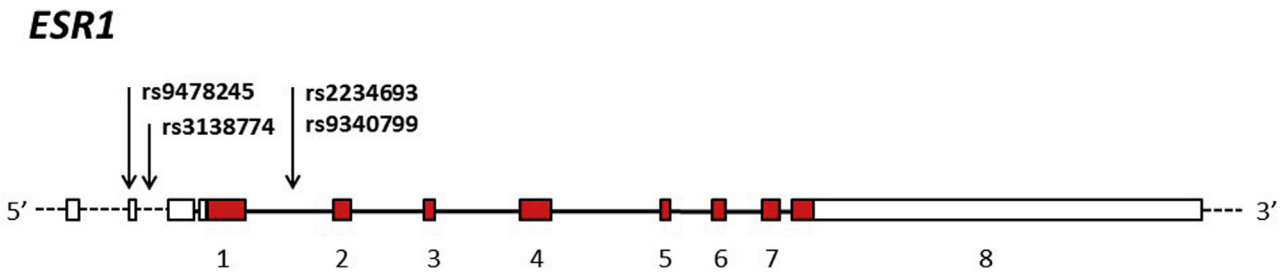

Figure 1. Adaptation of approximate positions of the analyzed polymorphisms in the *ESR1* gene. Boxes indicate exons, and lines indicate introns. Exon numbering is based on the primary transcripts as annotated in Ensembl (genome database project https://www.ensembl.org/index.html). The dotted lines and unnumbered exons indicate introns and exons of alternative transcripts. The arrows indicate the approximate positions of polymorphisms. Each polymorphism is labeled with the reference number (rs) assigned by NCBI (National Center for Biotechnology Information).

The exclusion criteria for all groups were: (1) presence of neurological or psychiatric disorders, (2) presence of hormonal disorders, (3) history of alcohol and/or drug abuse, (4) hospitalization for any reason in the 4 weeks before the evaluation, (5) having a severe medical or psychiatric disorder, (6) chromosome aneuploidy, inversions, and/or translocations.

To rule out the presence of psychiatric disorders and substance abuse within all transgender participants, the Mini-International Neuropsychiatric Interview[28] was administered.

Applying the first 5 criteria, we excluded 39 trans women and 1 trans man. We also excluded individuals with chromosome aneuploidy, inversions, and translocations (11 trans women and 8 trans men).[29] The final sample was made up of an extremely carefully chosen population of 273 trans women, 226 trans men, 252 cis women, and 285 cis men.

The study obtained the approval of the Ethics Committees of the Clínic Hospital, the Regional Universitario Hospital of Málaga and Universidad Nacional de Educación a Distancia (Madrid). All participants in the study signed an informed consent protocol beforehand.

Molecular Analysis

Genomic DNA was extracted from EDTA blood samples using the DNeasy Blood & Tissue Kit from Qiagen (Madrid, Spain). The analyzed polymorphisms were 3 single nucleotide polymorphisms (SNPs) C1 = rs9478245, C3 = rs2234693, and C4 = rs9340799; and one short tandem repeat polymorphism (STR): C2 = rs3138. According to the Ensembl database (www.ensembl.org/), C1 and C2 polymorphisms are located in the *ESR1* promoter, while C3 and C4 are located in the promoter flanking region (Table 1).

For SNPs (C1, C3, and C4), genotyping was performed by the overnight digestion of the PCR products with the corresponding digestion enzyme: *BsrDI* (Thermo Scientific, Madrid, Spain), *PvuII* (BioLabs, Madrid, Spain) or *XbaI* (Roche, Madrid, Spain), respectively. The digestion products were visualized in a 6% polyacrylamide electrophoresis gel (GE Healthcare, Madrid, Spain). The genotypes resulting from *BsrDI* and *PvuII* digestion were C/C, C/T, and T/T. And for *XbaI* were G/G, A/G, and A/A (Table 1).

For STR polymorphism (C2), genotyping was performed by automated capillary electrophoresis (3130 XL Genetic Analyzer, Applied Biosystems, Madrid, Spain), and allele length was determined by the GeneMapper-5 program (2012 Applied Biosystems, Madrid, Spain).

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, using SPSS 23.0 (IBM Corp, Armonk, NY, USA), considering significant a P value lower than 0.05.

For SNP polymorphisms (C1, C3, and C4), the allele and genotype frequencies were analyzed by χ^2 test. The strength of the associations with gender incongruence was measured by binary logistic regression, estimating the odds ratio (OR) for each genotype combination.

For STR polymorphism (C2), the mean number of repeats was analyzed by the Mann–Whitney U test. Subsequently, it was necessary to transform the C2 polymorphism into a dichotomous variable, short (S) vs long (L) alleles, taking as a cutoff the median obtained in the corresponding control group, resulting in the genotypes S/S (short-short), L/L (long-long), and S/L (short-long).

The 4 polymorphisms analyzed are located in the same chromosome, very close to each other (Table 1; Figure 1), and tend to be inherited together with a very high degree of correlation called linkage disequilibrium being observed. In addition, given that haplotypes may themselves be causal variants,[30] we were also interested in the simultaneous analysis of multiple loci (haplotypes).

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),[31] using logistic regression models to determine the strength of the associations. The false

Figure 2. The allele frequency of TA repeats for the C2 polymorphism in trans men and control cis women.

positives were controlled with the Bonferroni correction. In all analyses, a missing value for any response, polymorphism, or covariate was cause for exclusion of that individual from the analysis.

RESULTS

The final sample was made up of a highly homogeneous population of 273 trans women, 226 trans men, 252 cis women, and 285 cis men, after applying inclusion and exclusion criteria (39 trans women and 1 trans man were excluded), and excluding individuals with chromosome aneuploidy, inversions, and translocations (11 trans women and 8 trans men).

The polymorphisms were in Hardy-Weinberg equilibrium. The C2 allele distribution was consistently bimodal in all groups (Figures 2 and 3), with 2 peaks around 15 and 24 repeats. The number of repeats ranged from 10 to 28. When the mean number of repeats was analyzed by the Mann−Whitney *U* test, a difference was identified in the trans men population, having a significantly shorter median (16 repeats) with respect to cis women controls (18 repeats) ($Z = -2.035$, $P = .042$). The mean number of repeats between trans women (17 repeats) and cis men controls (18 repeats) was not significant ($Z = -0.610$, $P = .542$).

Analysis of the Allele and Genotype Frequencies by χ^2

We found significant differences in the distribution of the allele frequencies in the trans men vs cis women groups. We found over transmission of the C2 allele S and the C4 allele A in

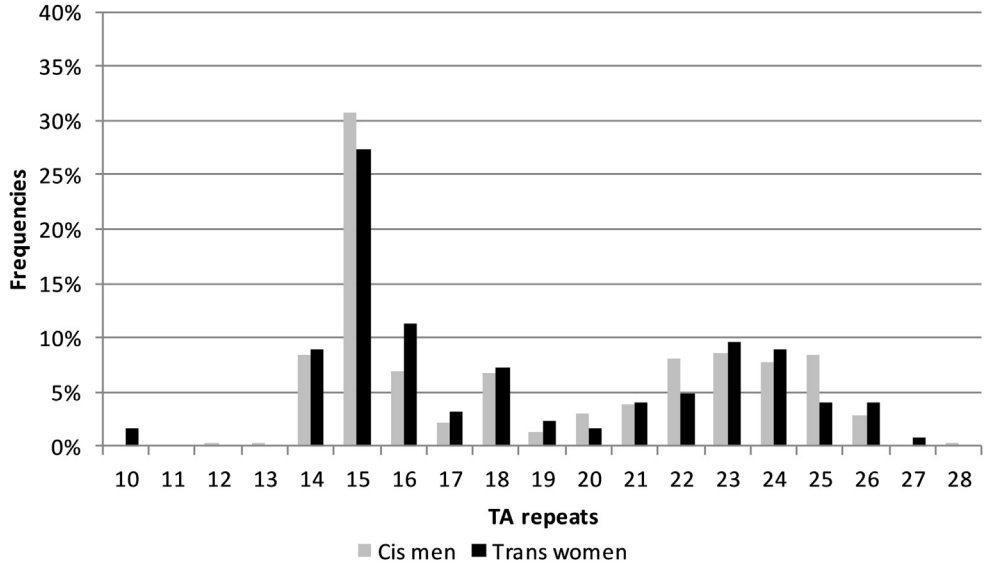

Figure 3. The allele frequency of TA repeats for the C2 polymorphism trans women and control cis men.

Table 2. C2 polymorphism association analysis with gender incongruence, in different models of inheritance

C2 association with transsexualism							
Model	Genotype	Cis groups (%)	Trans groups (%)	OR (95% CI)	*P*	AIC	BIC
Codominant	S/S	22.4	31.8	**1.00 (reference)**	–	572.3	588.8
	S/L	52	49.4	0.67 (0.42–1.06)	.07		
	L/L	25.7	18.8	0.52 (0.30–0.93)	.023*		
Dominant	S/S	22.4	31.8	**1.00 (reference)**	–	571.1	583.5
	S/L-L/L	77.6	68.2	0.62 (0.40–0.96)	.035*		
Recessive	S/S-S/L	74.3	81.2	**1.00 (reference)**	–	573.2	585.5
	L/L	25.7	18.8	0.68 (0.42–1.11)	.12		
Overdominant	S/S-L/L	48	50.6	**1.00 (reference)**	–	575.3	587.7
	S/L	52	49.4	0.89 (0.60–1.33)	.57		
Log-additive	—	—	—	0.72 (0.54–0.96)	.023*	570.4	582.8

OR = odds' ratio; AIC = Akaike's information criterion; BIC = Bayesian information criterion.
The risk for each genotype is compared with regards to the reference category **(1.00 reference).**
*Statistically significant ($P \leq .05$).

the trans men population ($\chi^2 = 5.175$; $P = .023$ and $\chi^2 = 4.952$; $P = .026$, respectively).

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 C2 and C4 polymorphisms showed significant differences for multiple patterns of inheritance (Tables 2 and 3). Genotypes S/S and S/L for C2 (Table 4) were genetic risk factors (OR>1) for the somatically female population [OR = 2.62 (1.23-5.58); $P = .012$ and OR = 2.32 (1.32-4.06); $P = .003$, respectively]. With respect to the C4 polymorphism (Table 5), the A/G genotype showed a genetic protective effect (OR<1) for the somatically female population [OR = 0.35 (0.16-0.77); $P = .009$] with respect to the A/A genotype.

Haplotype Analysis

The T allele for C1 was linked to the short (S) allele for C2, to the T allele for C3 and to the A allele for C4 (haplotype 1: T-S-T-A) (Table 6). However, the C allele for C1 was linked to the long (L) allele for C2, to the C allele for C3 and to the G allele for C4 (haplotype 6: C-L-C-G) (Table 6). The more frequent haplotypes were T-S-T-A (46.5%) and T-L-C-G (31.12%), while the haplotypes C-S-T-A and C-L-C-A (Table 6) were not present.

Haplotype interaction analysis with covariate "sex" (Table 7) showed that the haplotype 2: T-L-C-G represents a genetic protective factor [OR = 0.54 (0.35-0.83); $P = .005$] for individuals assigned as females at birth, and it is overrepresented in

Table 3. C4 polymorphism association analysis with gender incongruence in cis women and trans men, in different models of inheritance

C4 association with transsexualism							
Model	Genotype	Cis women (%)	Trans men (%)	OR (95% CI)	*P*	AIC	BIC
Codominant	A/A	31.7	55.2	**1.00 (reference)**	–	174.2	182.8
	A/G	53.3	32.8	0.35 (0.16–0.77)	.024*		
	G/G	15	11.9	0.46 (0.15–1.37)	.169		
Dominant	A/A	31.7	55.2	**1.00 (reference)**	–	172.5	178.1
	A/G-G/G	68.3	44.8	0.38 (0.18–0.78)	.007*,†		
Recessive	A/A-A/G	85	88.1	**1.00 (reference)**	–	179.4	185.1
	G/G	15	11.9	0.77 (0.28–2.14)	.61		
Overdominant	A/A-G/G	46.7	67.2	**1.00 (reference)**	–	174.2	179.9
	A/G	53.3	32.8	0.43 (0.21–0.88)	.019*		
Log-additive	—	—	—	0.57 (0.34–0.96)	.03*	175	180.6

OR = odds' ratio; AIC = Akaike's information criterion; BIC = Bayesian information criterion.
The risk for each genotype is compared with regards to the reference category **(1.00 reference).**
*Statistically significant ($P \leq .05$).
†Significant after the Bonferroni correction ($P < .05/4 = 0.0125$).

Table 4. Interaction analysis of the C2 polymorphism with covariate "sex"

Sex within polymorphism C2					
		Cis groups (%)	Trans groups (%)	OR (95% CI)	*P*
S/S	XY	8.95	3.93	**1.00 (reference)**	–
	XX	5.90	6.77	2.62 (1.23–5.58)	.012*,†
S/L	XY	20.31	6.33	**1.00 (reference)**	–
	XX	14.19	10.26	2.32 (1.32–4.06)	.003*,†
L/L	XY	9.83	3.06	**1.00 (reference)**	–
	XX	7.21	3.28	1.46 (0.62–3.44)	.393
Polymorphism C2 within sex					
		Cis groups (%)	Trans groups (%)	OR (95% CI)	*P*
XY	S/S	8.95	3.93	**1.00 (reference)**	–
	S/L	20.31	6.33	0.71 (0.36–1.42)	.333
	L/L	9.83	3.06	0.71 (0.31–1.60)	.421
XX	S/S	5.90	6.77	**1.00 (reference)**	–
	S/L	14.19	10.26	0.63 (0.33–1.19)	.158
	L/L	7.21	3.28	0.40 (0.18–0.88)	.023*

The risk for each genotype is compared with regards to the reference category **(1.00 reference).**
*Statistically significant ($P \leq .05$).
†Significant after the Bonferroni correction ($P < .05/4 = 0.0125$).

the cis women population; while haplotype 3: T-L-C-A is overrepresented in individuals assigned as females at birth [OR = 6.35 (1.35-29.96); $P = .019$] *vs* individuals assigned as males.

DISCUSSION

We detected an association between the estrogen receptor α gene *ESR1* promoter and gender incongruence, in the trans men population. Allele S (C2) and allele A (C4) were overrepresented and significantly associated with gender incongruence in our trans men population. Data from the C4 polymorphism corroborate our previous publication.[19,20]

Furthermore, haplotypes are also associated with gender incongruence. The haplotype 3: T-L-C-A was overrepresented in the trans men population, while the haplotype 2: T-L-C-G was overrepresented in cis populations. A theoretical analysis model of the *ESR1* promoter[32] predicted that the C1 (C) and C2 (L) minor alleles disrupted the transcription factor binding site for the sex-determining region on the Y chromosome, Sry-related HMG box-5 (SOX5), and the nerve growth factor-induced protein C (NGF1C), respectively. In addition, the C allele for C3 was predicted to remove the transcription factor binding sites for activating enhancer binding protein 4 (AP-4) in the sense strand, and the zinc finger protein ZNF238 (RP58) in the antisense strand.[32] Furthermore, C2 and C3 are located in a

Table 5. Interaction analysis of the C4 polymorphism with covariate "sex"

Sex within C4 polymorphism					
		Cis groups (%)	Trans groups (%)	OR (95% CI)	*P*
A/A	XY	52.27	37.25	**1.00 (reference)**	–
	XX	31.67	55.22	2.36 (1.04–5.36)	.039*
A/G	XY	36.36	45.10	**1.00 (reference)**	–
	XX	53.33	32.84	0.48 (0.21–1.11)	.084
G/G	XY	11.36	17.65	**1.00 (reference)**	–
	XX	15	11.94	0.49 (0.12-2.11)	.335
Polymorphism C4 within sex					
		Cis groups (%)	Trans groups (%)	OR (95% CI)	*P*
XY	A/A	52.27	37.25	**1.00 (reference)**	–
	A/G	36.36	45.10	1.74 (0.72–4.20)	.220
	G/G	11.36	17.65	2.18 (0.62–7.61)	.225
XX	A/A	31.67	55.22	**1.00 (reference)**	–
	A/G	53.33	32.84	0.35 (0.16–0.77)	.009*,†
	G/G	15	11.94	0.46 (0.15–1.37)	.17

The risk for each genotype is compared with regards to the reference category **(1.00 reference).**
*Statistically significant ($P \leq .05$).
†Significant after the Bonferroni correction ($P < .05/4 = 0.0125$).

Table 6. Haplotype frequencies estimation

Haplotypes								
	C1	C2	C3	C4	Total	Cis groups	Trans groups	Cumulative frequency
1	T	S	T	A	0.465	0.444	0.492	0.465
2	T	L	C	G	0.311	0.327	0.286	0.776
3	T	L	C	A	0.086	0.070	0.099	0.862
4	T	L	T	A	0.065	0.084	0.045	0.926
5	T	S	T	G	0.020	0.015	0.024	0.947
6	C	L	C	G	0.017	0.019	0.020	0.964
7	T	S	C	A	0.016	0.024	0.008	0.980
8	T	S	C	G	0.012	0.016	0.015	0.992
9	C	S	C	G	0.008	0	0.011	1
10	C	S	T	A	0	0	0	1
11	C	L	C	A	0	0	0	1

highly conserved region across species,[32] and according to Mooney, this enhances the possible implication of these 2 polymorphisms in the genetic basis (genetic vulnerability) of transgender incongruence.[33,34]

Taking into account these considerations of the biological importance of these polymorphisms,[35] our results suggest that C2 might be a functional polymorphism influencing *ESR1* expression regulation. However, these predictions need to be confirmed by transcription factor binding experiments.

In addition, C3 and C4 polymorphisms are located in the first intron of the *ESR1* gene and have a demonstrated significant effect on the level of protein synthesis.[36] Herrington et al[37] noted

Table 7. Haplotype and sex cross-classification interactions

Haplotypes						Population with XY karyotype		Population with XX karyotype	
	C1	C2	C3	C4	Frequency	OR (95% CI)	*P*	OR (95% CI)	*P*
1	T	S	T	A	0.468	**1.00 (reference)**	–	**1.00 (reference)**	–
2	T	L	C	G	0.306	1.01 (0.67–1.54)	.966	0.54 (0.35–0.83)	.005*,†
3	T	L	C	A	0.083	0.61 (0.22–1.69)	.347	2.53 (0.74–8.63)	.139
4	T	L	T	A	0.066	0.35 (0.07–1.83)	.209	0.43 (0.15–1.21)	.113
5	T	S	T	G	0.021	2.20 (0.39–12.50)	.379	–	–
6	C	L	C	G	0.019	–	–	0.37 (0.07–1.82)	.234
7	T	S	C	A	0.016	1.11 (0.13–9.64)	.931	–	–
8	T	S	C	G	0.014	–	–	0.66 (0.07–6.49)	.732
rare					0.006	–	–	–	–

Haplotypes						Population with XY karyotype	Population with XX karyotype	
	C1	C2	C3	C4	Frequency	OR reference	OR (95% CI)	*P*
1	T	S	T	A	0.468	**1.00 (reference)**	1.52 (0.83–2.80)	0.178
2	T	L	C	G	0.306	**1.00 (reference)**	0.81 (0.48–1.37)	0.439
3	T	L	C	A	0.083	**1.00 (reference)**	6.35 (1.35–29.96)	0.019*
4	T	L	T	A	0.066	**1.00 (reference)**	1.90 (0.33–11.02)	0.482
5	T	S	T	G	0.021	**1.00 (reference)**	–	–
6	C	L	C	G	0.019	**1.00 (reference)**	–	–
7	T	S	C	A	0.016	**1.00 (reference)**	–	–
8	T	S	C	G	0.014	**1.00 (reference)**	–	–
rare					0.006	**1.00 (reference)**	–	–

The risk for each haplotype is compared with regards to the reference category **(1.00 reference)**.
*Statistically significant ($P \leq .05$).
†Significant after the Bonferroni correction ($P < .05/4 = 0.0125$).

that the T/C variation for C3 results in a potential binding site for transcription factors that could enhance in vitro transcription. Thus, in some settings, the presence of the allele C (C3) might amplify *ESR1* transcription. Maruyama et al[38] also detected a small but significant enhancing activity that differed between haplotypes: enhancer activity by allele G (C4) was higher than the activity by allele A. Tangentially, pharmacogenetic studies on osteoporosis have suggested that women with haplotype C(C3)-G(C4) have a greater sensitivity to estrogen treatment than other women.[39]

In summary, data from cell-based assays, gene expression studies, and the fact that SNPs located in or near promoters have functional implications in gene expression[40,41] suggest that these polymorphisms might be functional SNPs influencing *ESR1* expression regulation. And thus, haplotypes T-L-C-A and T-L-C-G could be involved in the genetic vulnerability of gender incongruence in the trans men population.

In humans, the *ESR1* and *ESR2* genes encode the estrogen receptors ERα and ERβ, respectively, members of a family of nuclear receptor transcription factors, which are activated by estrogens. The 2 ERs exhibit different brain tissue distribution, exhibit different estrogen-binding affinities,[25] and also have distinct transcriptional properties.[42] Once bound by estrogens, the ER undergoes a dimerization (homodimer ERα-α, homodimer ERβ-β, or heterodimer ERα-β), allowing the receptor to interact with a greater or lesser affinity with specific DNA sequences located in or near promoter regions of target genes[43,44] and thereby modulate a transcription cascade process.[42,45] This capacity to directly regulate protein synthesis contributes to the potent and enduring effects of steroids on the developing brain.[22]

Estrogen is an important regulator of brain differentiation, and the ERs have a key function in sexual differentiation of brain and behavior.[46] In addition, ER α and β are found in both the developing[47] and adult human brain.[48]

If all these factors are taken into consideration, we could be lead to suppose that *ESR*1 promoter sequence variations (haplotypes) could modulate gene transcription, which, in turn, could modify important characteristics of the ERα receptor, modifying the receptor response to circulating estrogens during a prenatal "critical period", when the brain is sensitive to the organizational effects of testosterone and its metabolite estradiol.[22]

And because steroid hormones are among the most powerful and lasting signaling molecules in the body[22] and, in addition, a small variation in the receptor sensitivity could imply different effects on different brain tissues,[49] one might hypothesize that a small variation in the *ESR1* promoter sequence could implicate a great change in the brain.

One of the limitations of our work is that the analyzed sample is small and represents only a part of the trans population. As this sample was obtained from the population that attended the reference units requesting hormonal treatment, only a sample of trans people with dysphoria is analyzed in this paper. This makes our sample very homogeneous, as required for genetic analysis, but at the same time, it is only representative of a section of the global trans reality.[4]

In conclusion, our results have shown that the promoter of the *ESR1* gene is a candidate 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 interactions among steroids, steroids receptors, and multiple genes and polymorphisms. Exploration of estrogen receptors by methylation studies and also by microarray technology seems to be an interesting future focus in the study of sexual differentiation of the brain.

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