The (CA)n Polymorphism of *ERβ* Gene is Associated with FtM Transsexualism

Rosa Fernández, PhD,* Isabel Esteva, MD,† Esther Gómez-Gil, MD,‡ Teresa Rumbo, PhD*
Mari Cruz Almaraz, PhD,† Ester Roda, PhD,‡ Juan-Jesús Haro-Mora, PhD,† Antonio Guillamón, MD,§
and Eduardo Pásaro, PhD*

*Departamento de Psicobiología, Universidad de A Coruña, A Coruña, Spain; †Unidad de Transexualidad e Identidad de Género, Hospital Carlos Haya, Málaga, Spain; ‡Unidad de Identidad de Género, Hospital Clinic, Barcelona, Spain; §Departamento de Psicobiología, UNED, Madrid, Spain

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ABSTRACT

Introduction. Transsexualism is a gender identity disorder with a multifactorial etiology. Neurodevelopmental processes and genetic factors seem to be implicated.
Aim. The aim of this study was to investigate the possible influence of the sex hormone-related genes *ERβ* (estrogen receptor β), *AR* (androgen receptor), and *CYP19A1* (aromatase) in the etiology of female-to-male (FtM) transsexualism.
Methods. In 273 FtMs and 371 control females, we carried out a molecular analysis of three variable regions: the CA repeats in intron 5 of *ERβ*; the CAG repeats in exon 1 of *AR*, and the TTTA repeats in intron 4 of *CYP19A1*.
Main Outcome Measures. We investigated the possible influence of genotype on transsexualism by performing a molecular analysis of the variable regions of genes *ERβ*, *AR*, and *CYP19A1* in 644 individuals (FtMs and control females).
Results. FtMs differed significantly from control group with respect to the median repeat length polymorphism *ERβ* ($P = 0.002$) but not with respect to the length of the other two studied polymorphisms. The repeat numbers in *ERβ* were significantly higher in FtMs than in control group, and the likelihood of developing transsexualism was higher (odds ratio: 2.001 [1.15–3.46]) in the subjects with the genotype homozygous for long alleles.
Conclusions. There is an association between the *ERβ* gene and FtM transsexualism. Our data support the finding that *ERβ* function is directly proportional to the size of the analyzed polymorphism, so a greater number of repeats implies greater transcription activation, possibly by increasing the function of the complex hormone ERβ receptor and thereby encouraging less feminization or a defeminization of the female brain and behavior. **Fernández R, Esteva I, Gómez-Gil E, Rumbo T, Almaraz MC, Roda E, Haro-Mora JJ, Guillamón A, Pásaro E. The (CA)n polymorphism of *ERβ* gene is associated with FtM transsexualism. J Sex Med 2014;11:720–728.**

Key Words. Androgen Receptor; Aromatase; Estrogen Receptor; Gender Identity Disorder; Genetic Causes of Gender Identity Disorder; Transsexualism

Introduction

Female-to-male (FtM) transsexualism is characterized by persistent male identification and uneasiness with their assigned gender [1]. It is not possible to identify a single cause for transsexualism; rather, its origin seems to be multifactorial [2–5]. Recently, several studies have suggested that transsexualism might be associated with neurodevelopmental processes of the brain [6–11], while others imply the involvement of genetic factors [12–16].

Postmortem brain studies have shown that the volume and the number of neurons of the central part of the bed nucleus of the *stria terminalis* and the third interstitial nucleus of the anterior hypothalamus (INAH3) are feminized in male-to-female transsexuals (MtFs) [6,17]. A similar pattern was reported in the INAH3 of FtMs [18]. Recently, the gray and white matter regions of the brain of FtMs were studied before and after cross-sex hormonal treatment. The white matter microstructure pattern in untreated FtMs was closer to males than to females; before testosterone

treatment, they presented a female phenotype with a masculine and/or defeminized profile in brain bundles that are related to complex cognitive function [19]. Only these bundles respond to androgenization, and they do so in the way that males respond [20]. In relation to the gray matter, untreated FtMs showed evidence of subcortical gray matter masculinization; they presented a masculinization of their right putamen. However, their cortical thickness did not differ from control females but it was greater than in males in the parietal and frontal cortices [7]. Luders et al. [10] analyzed magnetic resonance imaging (MRI) data of 24 MtFs not yet treated with cross-sex hormones to determine whether gray matter volumes in MtFs more closely resemble people who share their biological sex or people who share their gender identity. The results revealed that regional gray matter variation in MtFs is more similar to the pattern found in men than in women. However, MtFs show a significantly larger volume of regional gray matter in the right putamen compared with men. These findings provide new evidence that transsexualism is associated with a distinct cerebral pattern, which supports the assumption that brain anatomy plays a role in gender identity [10].

The involvement of genetic factors in transsexualism has come mainly from familial studies [21,22], familial cases of twins being concordant for transsexualism [12,23], and from molecular genetic studies of certain polymorphisms of androgen and estrogen system genes [13–15,24,25].

The androgen receptor (AR) is implicated in the differentiation of the cortical cortex. The possession of an allele with a smaller number of CAG repeats confers more efficient functioning of the receptor and is associated with "masculinization" of the cortex in adolescence [26].

For the estrogen receptor (ER), two subtypes, the alpha ERα and beta ERβ, have been identified [27]. Expression of the beta subtype is clearly higher in several brain regions [28], and male mice lacking functional ERβ have an incompletely defeminized brain and behavior [29]. ERα is primarily involved in masculinization, while ERβ has a major role in defeminization of sexual behaviors [29].

Moreover, animal studies have clearly demonstrated that prenatal exposure to testosterone plays a primary role in neural and behavioral sexual differentiation [30]. Testosterone binds to and activates ARs and is converted to estrogen by aromatase (CYP19A1) in the brain, and consequently activates the central ERα and ERβ. It may cause masculinization directly by activation of AR or indirectly by activation of ERs [31,32]. Hence, the genes coding for the *ERβ*, *AR*, and *CYP19A1* are reasonable candidates in the quest for genes that may influence the likelihood of developing transsexualism.

Previous studies analyzing these genes have found discordant results [13–15]. In a work with 29 FtMs, Henningsson et al. found that FtMs differed from controls with respect to the median length of the *ERβ* repeat polymorphism but not with respect to the length of the other two studied polymorphisms. Hare et al., in 112 MtFs, have found a significant association between longer *AR* gene polymorphisms and transsexualism. Finally, Ujike et al., in a study performed on 168 FtMs and 74 MtFs, found no significant differences.

The aim of our study was to investigate the possible influence of the sex hormone-related genes *ERβ*, *AR*, and *CYP19A1* in the etiology of transsexualism by performing a molecular analysis of the variable regions of these genes in 273 FtMs and 371 control females.

Method

Subjects

The subjects comprised 273 FtMs and 371 age and geographical origin-matched control females. The selection of FtMs was conducted through both the Andalusian Gender Identity Unit (Carlos Haya Hospital of Málaga) and the Gender Identity Units of Catalonia (Clínic Hospital of Barcelona). The diagnoses were made using the *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition, text revised) (American Psychiatric Association, 2000) and the International Classification of Diseases-10 criteria [33–35]. We excluded patients with major psychiatric illnesses such as schizophrenia or bipolar disorders. All patients received medical examinations by an endocrinologist to rule out the anomaly of the external genitalia and internal sex organs.

All participants were characterized by early-onset gender nonconformity (before puberty), erotic attraction to females, right-handedness in writing, and good physical health. Participants had no endocrine, neurological, or major psychiatric comorbidity.

Sociodemographic, clinical, and psychiatric data that included any family background of transsexuality were completed for all patients as

Table 1 Description of the polymorphic regions analyzed

Gene	Position	Tandem repeat	Primers	Conditions of amplification
ERβ	14q22–24	CA intron 5	5′-AACAAAATGTTGAATGAGTGGG-3′ 5′-GGTAAACCATGGTCTGTACC-3′ FAM	35 cycles: 92°C 40 seconds 57°C 40 seconds 72°C 40 seconds 72°C 10 minutes
AR	Xq11–12	CAG exon 1	5′-GTTCCTCATCCAGGACCAGGTA-3′ 5′-GTGCGCGAAGTGATCCAGA-3′ HEX	35 cycles: 92°C 1 minute 56°C 1 minute 72°C 1 minute 72°C 10 minutes
CYP19A1	15q21	TTTA intron 4	5′-TTACAGTGAGCCAAGGTCGT-3′ 5′-GCAGGTACTTAGTTAGCTAC-3′ NED	35 cycles: 92°C 1 minute 58°C 1 minute 72°C 1 minute 72°C 10 minutes

part of similar standard clinical assessments at both clinics [36–38].

The control group consisted of a random group of individuals from a Spanish population, previously used in metabolic and genetic studies, age and sex adjusted with the FtM group [39,40]. They were free of any neurological, systemic, or psychiatric illness, as verified by a detailed interview. An inclusion criterion for all participants was to be free of psychotropic medication and/or illegal drug use. The study only included heterosexual controls.

The study was initiated after obtaining approval from the Ethics Committees of the University of A Coruña, Clínic Hospital (Barcelona), and Carlos Haya Hospital (Málaga). We drafted a protocol and obtained written, informed consent from each of the participants in the study.

Genetic Analysis

Cytogenetic Analyses

Peripheral blood samples were extracted for cytological and molecular analyses. Chromosomes were prepared according to standard techniques from peripheral blood, [41] and the preparations were treated with trypsin to obtain G-banding [42]. Patients with chromosomal aberrations like translocations, inversions, or chromosome number aberration were discarded.

Genotype Assessment by High-Density (HD) Array

Genomic DNA was extracted using the DNeasy Blood & Tissue Kit from Qiagen (Madrid, Spain). The genome-wide DNA copy number analyses were performed with CytoScan™ HD Array (Affymetrix, Madrid, Spain) in accordance with the manufacturer's instructions.

Molecular Analyses

The polymorphic regions were amplified by polymerase chain reaction following the protocol outlined in Table 1. All genotyping was performed in a blinded fashion,and in triplicate in case of failure reactions. Finally, the fragments were analyzed by automated capillary electrophoresis 3130 XL Genetic Analyzer from Applied Biosystems (Madrid, Spain).

Statistical Analysis

Independent samples were analyzed by taking the medians of Mann–Whitney *U* and chi-square tests,using the median length of the two alleles, short (S) and long (L). To calculate the cutoff point to differentiate between S and L alleles, we took into account the median of the polymorphisms of individual genes.

Analyses were performed using SPSS 17.0 software (SPSS, Chicago, IL, USA). A P value ≤ 0.05 was considered significant. Interactions between the three polymorphisms were evaluated using a binary logistic regression model.

Results

The analysis by HD arrays allowed us to examine the karyotype at the molecular level. Eleven patients from the FtM group were excluded for small pericentromeric inversions or translocations. We were unable to find any karyotypic alteration specific to the FtM transsexualism because genetic

variants found in the FtM group also appeared in control females, and therefore are not considered variations with clinical significance.

With respect to the analysis of polymorphisms, the FtM group showed 16 alleles for *ERβ* (Figure 1A), in a range of repeats between 19–35 (>90% alleles have 22 to 30 CA repeats); 19 different alleles for the *AR* gene (Figure 1B), the number of repeats extending between 7 and 28 (>90% alleles have 16–24 CAG repeats); and 11 alleles for the *CYP19A1* gene (Figure 1C) and 4–15 repeats with a characteristic "U" distribution.

In the control female group, the molecular analysis showed 14 alleles for the *ERβ* gene extending between 18 and 30 repeats (Figure 1A), the *AR* gene showed 15 alleles between 12 and 26 repeats (Figure 1B), and the gene *CYP19A1* showed 10 alleles between 4 and 14 repeats (Figure 1C) with the same characteristic "U" distribution. The percentages of each allele are shown in Figure 1.

Significant differences were found only in the *ERβ* gene when we compared the number of repeats in both populations, with a higher value in the FtM group. The *P* value obtained in the Mann–Whitney *U*-test was 0.002 (Table 2).

To calculate the allele frequencies, we differentiated between S and L alleles, taking into account the median of the polymorphisms for individual genes. So for the *ERβ* gene, the difference between S and L alleles remained at 26 repeats, for the *AR* gene at 19 repeats, and for *CYP19A1* at 8 repeats (Table 3). After this separation, no significant differences were found in the distribution of L and S alleles for *AR* or *CYP19A1*, but they were significant for the *ERβ* gene ($P = 0.001$ for the chi-square test) (Table 3). The odds ratio (OR) data for the allele frequency show significant values for the L allele vs. the S: OR = 1.508 with confidence intervals at 95% (1.777–1.911).

In a second step, the lengths of the three polymorphisms were subclassified to obtain genotype frequencies. The genotypes were determined as SS, LL, and heterozygous SL (Table 4). The data showed a significant association between the phenotype and the *ERβ* gene, $P = 0.001$, for the chi-square test. For all other variables, no significant association was established (Table 4).

The OR data indicate a significant association only for the genotype LL vs. SS: OR = 2.001 with confidence intervals at 95% (1.154–3.464), establishing that the probability of the FtM transsexualism is greater for the genotype LL compared with SS.

Finally, we applied a binary logistic regression model. The three variables and all the possible interactions among them were included in the model. We used the Wald statistic to evaluate the significance of the model coefficients (Table 5). The model fits the data based on the values of –2LL (701.761) and Cox-Snell R^2 (0.095) and Nagelkerke (0.129). None of these interactions were statistically significant.

Discussion

We investigated the possible influence of the sex hormone-related genes *ERβ*, *AR*, and *CYP19A1* on the etiology of FtM transsexualism by performing a molecular analysis of the variable regions (CA repeats in *ERβ*, CAG repeats in *AR*, and TTTA repeats in *CYP19A1*) in 273 FtMs and 371 control females. To the best of our knowledge, this is the largest group of FtMs analyzed so far.

We grouped the data into S and L alleles according to the median repeat length polymorphism, obtaining the individual's genotype (SS, SL, or LL) for the three genes. FtMs differed from the control group with respect to the median length of the *ERβ* polymorphism but not with respect to the length of the other two studied genes. Considering the data for categorical variables of S and L alleles, and the genotypes SS, SL, and LL, we found significant *P* values for *ERβ* gene and genotype frequencies but not for *AR* and *CYP19A1* genes. A greater number of CA repeats corresponds to greater probabilities of FtM transsexualism.

Our data complement the previous study on transsexualism from Ujike et al. [17] who examined the same three polymorphisms (*ERβ*, *AR*, and *CYP19A1*) in a transsexual population. These authors did not find any association between the three polymorphisms and transsexualism nor any interactions between the genetic variables. Moreover, there are another two works addressing the molecular analysis of transsexualism: Hare et al. [13] and Henningsson et al. [15]. Both are similar studies but in an MtF population.

To the best of our knowledge, this is the first time that a technique of genotype assessment by HD arrays has been applied to research transsexualism. This allowed us to perform an exhaustive analysis of any type of small chromosomal alteration. So using this new advanced technique of molecular karyotyping and looking for the homogeneity of the sample analyzed, we excluded any chromosome alteration that may be disturbing the results. Moreover, following the

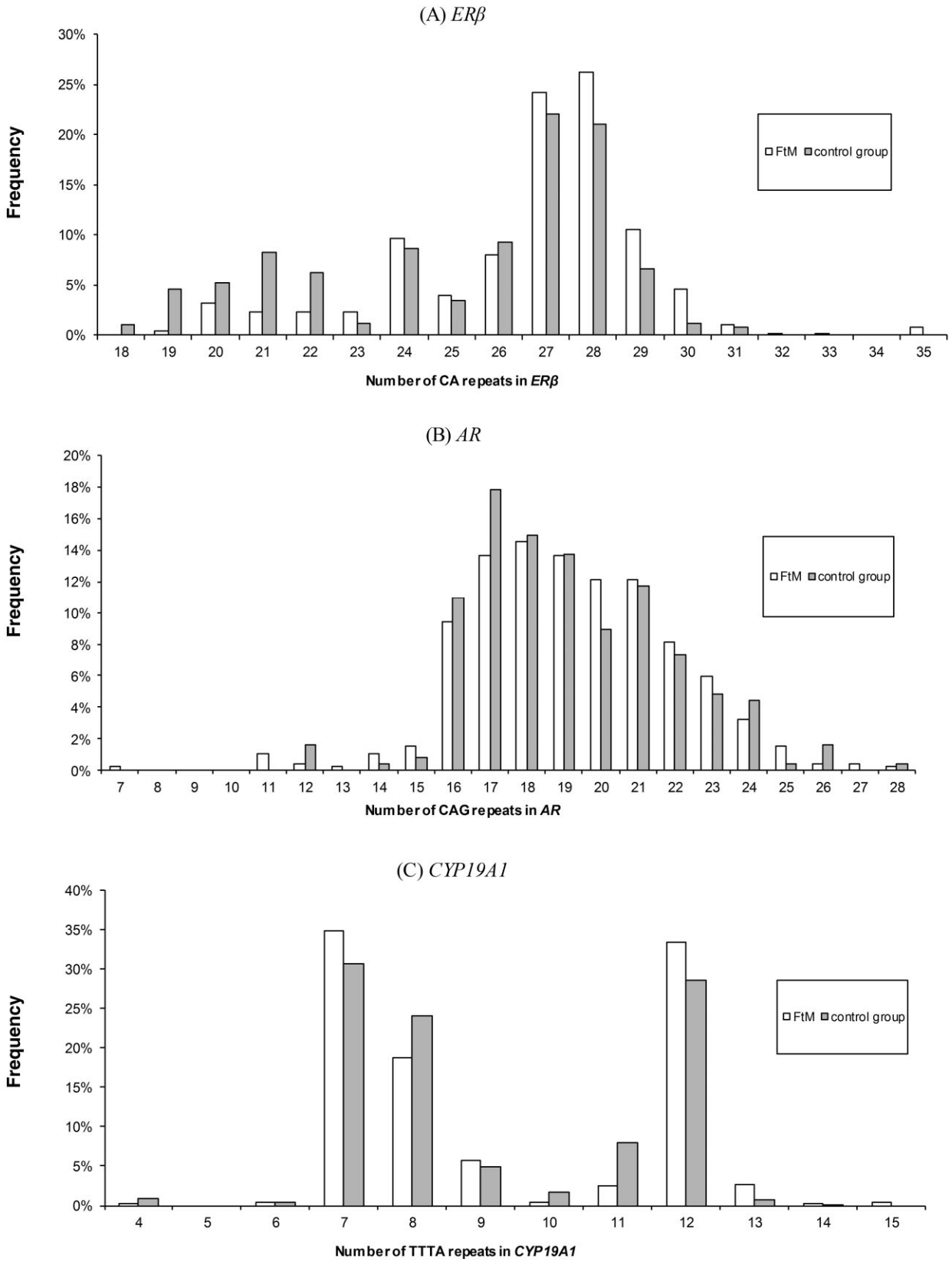

Figure 1 Distribution of allele frequency. FtM = female-to-male

Table 2 Dates of the median data of tandem repeats of *ERβ*, *AR*, and *CYP19A1* in FtM and control groups

Gene	Group	N	Mean of tandem repeats	SD	Mann–Whitney *U*-test	*P* value
ERβ	Control group	371	25.56	1.90	31918	0.002[a]
	FtM group	238	26.83	1.86		
AR	Control group	365	19.27	1.88	40992	0.402[b]
	FtM group	236	19.45	2.19		
CYP19A1	Control group	371	9.33	1.56	42524	0.160[b]
	FtM group	245	9.51	2.24		

[a]Significant differences with respect to control group; $P \leq 0.05$
[b]Nonsignificant differences with respect to control group; $P > 0.05$
FtM = female-to-male; SD = standard deviation

Table 3 Data of the allele frequencies for *ERβ*, *AR*, and *CYP19A1* after division into short and long alleles

Gene	Allele	Frequencies FtM group	Frequencies Control group	Chi-square test	*P* value
ERβ	Short (<26)	150 (31.51%)	303 (40.84%)	9.759	0.001[a]
	Long (≥26)	326 (68.49%)	439 (59.16%)		
Total		476 (100%)	742 (100%)		
AR	Short (<19)	253 (53.6%)	374 (44.38%)	1.618	0.203[b]
	Long (≥19)	219 (46.4%)	376 (55.62%)		
Total		472 (100%)	730 (100%)		
CYP19A1	Short (≤ 8)	285 (52.2%)	416 (56.06%)	0.53	0.466[b]
	Long (>8)	205 (41.8%)	326 (43.94%)		
Total		490 (100%)	742 (100%)		

[a]Significant differences with respect to control group; $P \leq 0.05$
[b]Nonsignificant differences with respect to control group; $P > 0.05$
FtM = female-to-male

same assumption of homogeneity, all subjects (FtMs and control group) were of Spanish origin.

In the case of the *AR* and *CYP19A1* genes, we did not find any relationship between the genes and FtM transsexualism. However, in the case of exon 5 of the *ERβ* gene, and contrary to that described by Ujike et al. [14], we found a direct relationship between the length of the variable region and FtM transsexualism, so the greater the number of repeats, the greater the susceptibility to transsexualism.

Although there are numerous studies showing the inverse relationship between the length of the *AR* gene and the activity of the hormone-receptor complex [43–45], there are no data indicating that this same inverse relationship exists in the case of *ERβ*. Some works bear on this possibility; Kudwa et al. [31] found that male mice lacking functional *Erβ*, when treated with the appropriate hormonal priming, display significantly more female-like sexual receptivity than littermates. Yet, lack of functional ERβ receptors does not impair normal

Table 4 Frequencies of the genotypes for *ERβ*, *AR*, and *CYP19A1* after division into SS, SL, and LL genotypes

Gene	Genotype	Frequencies FtM group	Frequencies Control group	Chi-square test	*P* value
ERβ	SS	24 (10.08%)	51 (13.75%)	13.086	0.001[a]
	SL	102 (42.86%)	201 (54.18%)		
	LL	112 (47.11%)	119 (32.08%)		
Total		238 (100%)	371 (100%)		
AR	SS	73 (30.9%)	101 (26.93%)	1.497	0.473[b]
	SL	107 (45.3%)	172 (45.87%)		
	LL	56 (23.7%)	102 (27.20%)		
Total		236 (100%)	365 (100%)		
CYP19A1	SS	79 (32.2%)	113 (30.46%)	0.656	0.720[b]
	SL	127 (51.8%)	190 (51.21%)		
	LL	39 (15.09%)	68 (18.33%)		
Total		245 (100%)	371 (100%)		

[a]Significant differences with respect to control group; $P \leq 0.05$
[b]Nonsignificant differences with respect to control group; $P > 0.05$
FtM = female-to-male; L = long alleles; S = short alleles

Table 5 Binary logistic regression analyses of gene–gene interactions for susceptibility to FtM transsexualism

Gene	df	Wald	*P* value
ERβ	1	11.626	0.001[a]
AR	1	3.223	0.073[b]
CYP19A1	1	1.337	0.248[b]
AR by *ERβ*	2	0.716	0.397[b]
AR by *CYP19A1*	2	0.296	0.586[b]
CYP19A1 by *ERβ*	2	0.076	0.783[b]
AR by *CYP19A1* by *ERβ*	3	0.523	0.470[b]

[a]Significant; $P \leq 0.05$
[b]Not significant; $P > 0.05$
FtM = female-to-male

expression of adult masculine sexual behavior. They found no evidence showing that masculinization is deficient in ERβKO males (rats genetically modified without the *Erβ* gene); however, they propose that the defeminization process is incomplete in ERβKO males. Our data, like previous studies [29,46], support the finding that a functioning ERβ receptor is directly proportional to the size of the analyzed polymorphism, so a greater number of repeats implies greater transcription activation, therefore, an increase in ERβ receptor function, and finally, an increase in defeminization in females. Thus, one could propose that the greater efficiency of the estrogen-receptor complex by a high number of repeats would lead to a reduction in feminization, favoring a defeminization process [47]. Defeminization of the corticospinal tract has been described in FtMs [19].

Westberg et al. [46] found that women with relatively few CA repeats of the *ERβ* gene displayed higher testosterone levels and lower sex steroid hormone-binding globulin levels than those with many CA repeats. The apparent association between a short CA repeat region of the *ERβ* gene and high levels of testosterone suggests that this variant of the gene leads to a less active receptor [48]. Needless to say, a detailed discussion of the possible mechanisms underlying the apparent association between the *ERβ* gene polymorphism studied and the hormonal activity must await further clarification of the influence of this polymorphism on receptor function.

The present study has several strengths. To the best of our knowledge, this is the first time that a technique of HD arrays has been applied to research transsexualism. This technique allows us to exclude any chromosome alterations that may be disturbing the results. Furthermore, this study has one of the largest sample sizes analyzed so far.

This study also has some limitations. First, because the subjects were of Spanish origin, the results cannot be generalized to all populations. That could be the reason for the differences between our results and those of Ujike et al. [14]. Second, similar to the work of Ujike et al. [14] and in contrast to that indicated by Hare et al. [13] and Henningsson et al. [15], when we applied the binary logistic regression model, none of the interactions were statistically significant. And finally, the study was performed only in FtMs. Therefore, similar studies on MtF transsexuals in the same population are needed.

Conclusions

Our data support the association between the *ERβ* gene and FtM transsexualism. The higher number of CA repeats implies greater transcription activation, and therefore, also lower feminization or a greater defeminization. Thus, the susceptibility to transsexualism was higher in the subjects with genotype homozygous LL.

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Corresponding Author: Eduardo Pásaro, MD, Departamento de Psicología, Area Psicobiología, Universidad de A Coruña, Campus Elviña, 15071 A Coruña, Spain. Tel: +34(981) 16-7000; Fax: +34(981) 16-7115; E-mail: eduardo.pasaro@udc.es

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Statement of Authorship

Category 1

(a) Conception and Design
Eduardo Pásaro; Rosa Fernández; Isabel Esteva; Esther Gómez-Gil; Mari Cruz Almaraz; Antonio Guillamón

(b) Acquisition of Data
Rosa Fernández; Teresa Rumbo; Ester Roda; Juan-Jesús Haro-Mora

(c) Analysis and Interpretation of Data
Eduardo Pásaro; Rosa Fernández; Isabel Esteva; Esther Gómez-Gil; Antonio Guillamón

Category 2

(a) Drafting the Article
Eduardo Pásaro; Rosa Fernández; Isabel Esteva; Esther Gómez-Gil; Antonio Guillamón

(b) Revising It for Intellectual Content
Eduardo Pásaro; Isabel Esteva; Esther Gómez-Gil; Antonio Guillamón

Category 3

(a) Final Approval of the Completed Article
Eduardo Pásaro; Rosa Fernández

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