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Molecular basis of Gender Dysphoria: androgen and estrogen receptor interaction

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ABSTRACT

Background: Polymorphisms in sex steroid receptors have been associated with transsexualism. However, published replication studies have yielded inconsistent findings, possibly because of a limited sample size and/or the heterogeneity of the transsexual population with respect to the onset of dysphoria and sexual orientation. We assessed the role of androgen receptor (AR), estrogen receptors alpha (ERα) and beta (ERβ), and aromatase (*CYP19A1*) in two large and homogeneous transsexual male-to-female (MtF) and female-to-male (FtM) populations.

Methods: The association of each polymorphism with transsexualism was studied with a twofold subject-control analysis: in a homogeneous population of 549 early onset androphilic MtF transsexuals *versus* 728 male controls, and 425 gynephilic FtMs *versus* 599 female controls. Associations and interactions were investigated using binary logistic regression.

Results: Our data show that specific allele and genotype combinations of ERβ, ERα and AR are implicated in the genetic basis of transsexualism, and that MtF gender development requires AR, which must be accompanied by ERβ. An inverse allele interaction between ERβ and AR is characteristic of the MtF population: when either of these polymorphisms is short, the other is long. ERβ and ERα are also associated with transsexualism in the FtM population although there was no interaction between the polymorphisms. Our data show that ERβ plays a key role in the typical brain differentiation of humans.

Conclusion: ERβ plays a key role in human gender differentiation in males and females.

1. Introduction

Transsexualism in ICD-10 (World Health Organization, 1993), Gender Identity Disorder in DSM-IV-TR (American Psychiatric Association, 2000), Gender Dysphoria in DSM-5 (American Psychiatric Association, 2013) or Gender Incongruence in ICD-11 (World Health Organization, 2018) are characterized by a marked incongruence between one´s experienced gender and biological sex (World Health Organization, 1993). Transsexuals are individuals who seek, or have undergone, a social transition from male-to-female (MtF) or female-to-male (FtM) that in many, but not all cases involves a somatic transition through cross-sex hormone treatment and genital surgery (American Psychiatric Association, 2013). One way to approach the study of factors contributing to gender development is to compare MtF and FtM individuals to others with typical gender identification.

A review of the genetic literature on transsexualism has shown that concordance is higher in monozygotic than in dizygotic twins among both MtF and FtM individuals (Heylens et al., 2012), pointing to a

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genetic basis underlying transsexual development. In addition, differences have been reported by studies of genetic polymorphisms in steroid receptors and enzymes implicated in the sexual differentiation of brain (Henningsson et al., 2005; Hare et al., 2009; Ujike et al., 2009; Fernández et al., 2014a,b, 2016; Cortés-Cortés et al., 2017). Differences were also reported in anatomical *post mortem* (Swaab, 2004) and *in vivo* brain studies (Guillamón et al., 2016). Moreover, MRI (Magnetic Resonance Imaging) studies show differing brain morphologies in MtF, FtM, females and males, reflecting different brain phenotypes that may originate in atypical developmental effects produced by sex hormones in specific cortical regions (Guillamón et al., 2016) and subcortical structures (Zhou et al., 1995).

The biological actions of sex steroids are mediated by binding to specific nuclear receptors that are members of an extended family of transcription factors. The ligand–receptor complex translocates to the nucleus and promotes sex-specific gene expression (Matthews and Gustafsson, 2003). The direct induction of gene expression *via* activation of the estrogen receptors (ERs) α and β and the androgen receptor (AR) is the presumptive route for brain masculinization (Sato et al., 2004; Kudwa et al., 2006).

In lower mammals ERα is primarily involved in masculinization, while ERβ has a major function in defeminization of sexual behavior (Kudwa et al., 2006). In rodents, estradiol induces two independent developmental processes: masculinization of neural circuits that will support male-typical reproductive behaviors in adults and defeminization, the loss of the ability to display typical adult female behavior, which is also an active developmental process (McCarthy, 2008). However, it is believed that in non-human primates (Wallen, 2005), as well as in humans (Swaab, 2004), estrogenic metabolites from androgens are not critical to masculinization and defeminization (Wallen, 2005).

All these observations have led to the study of the involvement of DNA polymorphisms of ERβ, ERα, AR, and the aromatase (*CYP19A1*) in transsexuality (Henningsson et al., 2005; Hare et al., 2009; Ujike et al., 2009; Fernández et al., 2014a,b, 2016; Cortés-Cortés et al., 2017). However, the reported results have been inconsistent or negative (Meyer-Bahlburg, 2011). The lack of agreement between different publications might be due to the small samples studied and/or the heterogeneity of the transsexual population in relation to the onset of the gender dysphoria (*i.e.* before or after puberty) and sexual orientation.

In order to address all these questions, this work studied the implication of the polymorphisms (CA)n-ERβ (rs113770630), XbaI-ERα (rs9340799), (CAG)n-AR (rs193922933) and (TTTA)n-CYP19A1 (rs60271534) in a large and homogenous sample of 549 early onset androphilic MtFs *vs* 728 male controls and 425 early onset gynephilic FtMs *vs* 599 female controls. The analyses were conducted independently for a somatically[1] female population (FtM *vs* female controls) and a somatically male population (MtF *vs* male controls).

Moreover, because it is unknown whether androgen and estrogen genotypes interact with each other in the genesis of gender, we also analyzed the cross interactions between the AR polymorphism and the other above-mentioned polymorphisms (ERβ, ERα and CYP19A1).

2. Methods and materials

2.1. Participants

The initial population was 599 MtFs, 434 FtMs, and 599 female and 728 male controls recruited through the Gender Units of the Hospital Clínic of Barcelona (Spain) and the Hospital Carlos Haya of Málaga (Spain). As we began collecting the sample in 2010, during the first three years transsexuals were diagnosed using the DSM-IV-TR (American Psychiatric Association, 2000) (Gender Identity Disorder in Adolescent or Adults 302.85), which is focused on gender identity *per se*, but, since 2014, we have used the DSM-5 (American Psychiatric Association, 2013) [Gender Dysphoria (GD) in Adolescents and Adults, 302.85], which focuses on dysphoria as the clinical problem. However, all subjects in our study fulfill the definition of transsexuals stated in DSM-IV-TR: all felt a strong and persistent identification with the other sex, and expressed a strong desire for a social transition from male to female or female to male that involved somatic transition by cross-sex hormone treatment and, in most, genital surgery. The initial exclusion criteria were head trauma, neurological disorder, major medical condition and history of alcohol and/or drug abuse. We applied three inclusion criteria to this population: early onset of GD (before puberty), being androphilic (MtF) or gynephilic (FtM) and aged between 18 and 47 years old at enrollment. With these criteria 39 MtFs and 1 FtM were excluded. We also excluded individuals with chromosome aneuploidy, inversions and translocations (11 MtFs and 8 FtMs). Sociodemographic and clinical characteristics from a transsexual Spanish sample of similar characteristics have been described in detail elsewhere (Gómez-Gil et al., 2009).

Male and female control groups were described in a previous study (Soriguer et al., 2013). They were selected randomly from a country census (Pizarra, Málaga, Spain). The inclusion age was 18–65 years. Individuals were excluded if they had been hospitalized for any reason in the four weeks prior to the evaluation, were pregnant, in a geriatric institution, or had a severe medical or psychiatric disorder (these exclusion criteria were also applied to the transsexual population). The age and sex sample distribution was not different from the general population distribution (Soriguer et al., 2013). The controls were free of chromosomal anomalies. The final sample of the study was made up of 549 MtFs, 425 FtMs, and 599 female and 728 male controls.

The study was initiated after obtaining approval from the Ethics Committees of the Clínic Hospital, Carlos Haya Hospital, Universidad de A Coruña, and Universidad Nacional de Educación a Distancia (Madrid).

2.2. Cytogenetic analysis

Chromosomes were prepared according to standard techniques from peripheral blood (Moorhead et al., 1960) to obtain G-banding karyotypes (Seabright, 1971).

2.3. Molecular analysis

Genomic DNA was extracted from EDTA blood samples using the DNeasy Blood & Tissue Kit from Qiagen (Madrid, Spain). Polymorphisms were selected for study on the basis of their known implication in the development of cerebral sex differences in humans (Raznahan et al., 2010) and had already been studied by ourselves and others (Henningsson et al., 2005; Hare et al., 2009; Ujike et al., 2009; Fernández et al., 2014a,b, 2016; Cortés-Cortés et al., 2017). The polymorphisms selected for each gene were as follows, for the *ESR2* gene: (CA)n-ERβ (rs113770630); for the *ESR1* gene: XbaI-ERα (rs9340799); for the *AR* gene: (CAG)n-AR (rs193922933); and for the aromatase *CYP19A1* gene: (TTTA)n-CYP19A1 (rs60271534) (Supplemental Figure S1). These polymorphic regions were amplified by PCR following the previously outlined protocols (Fernández et al., 2014a,b; Cortés-Cortés et al., 2017) (Supplemental Table 1).

Three of the polymorphisms analyzed were short tandem repeat polymorphisms (STRs): (CA)n-ERβ, (CAG)n-AR and (TTTA)n-CYP19A1; and one was a single nucleotide polymorphism (SNP): XbaI-ERα. In the case of tandem polymorphisms, genotyping was performed by automated capillary electrophoresis (3130 XL Genetic Analyzer, Applied Biosystems, Spain), and allele length was determined by the GeneMapper-5 program (2012 Applied Biosystems, Spain). In the case

[1] With respect to FtMs and MtFs, the use of the word “somatically” refers to individuals natally assigned as females or males respectively.

of the XbaI-ERα polymorphism, genotyping was performed by the overnight digestion of the PCR product with the enzyme *Xba*I (Roche, Spain) (Supplemental Table 1) and visualized in a 6% polyacrylamide non-denaturing electrophoresis gel (GE Healthcare, Spain). The genotypes resulting from digestion with *Xba*I were G/G, A/G, and A/A.

2.4. Statistical methods

First, to evaluate for possible associations with transsexualism, comparisons between MtF *vs* male controls and FtM *vs* female controls were performed: for the repeat length of the STR polymorphisms applying Mann-Whitney test, and for the SNP polymorphism with the allele and genotype frequencies and Chi-squared test, using SPSS® 23.0 software.

Second, to seek a combined effect of the polymorphisms ERβ, ERα, AR and CYP19A1 in transsexualism, a cross-interaction analysis was carried out with a binary logistic regression model, separately in two independent populations: somatically females (FtM *vs* female controls) and somatically males (MtF *vs* male controls). The AR gene is located at the X chromosome, so 46,XY individuals have only one allele. For that reason, the AR polymorphism was fixed, fitting the logistic models for each AR allele separately. The analyses were performed using the free online software SNPStats, http://bioinfo.iconcologia.net/SNPstats (Solé et al., 2006), estimating the odds ratio (OR) for each genotype combination with respect to the reference.

For the cross-interaction analyses it was necessary to transform the tandem polymorphisms into dichotomous variables, short (S) *vs* long (L) alleles, taking as a cutoff the median obtained in the corresponding control groups (ERβ: short ≤ 26.66 for males and short ≤ 26.61 for females; AR: short ≤ 18.55 for males and short ≤ 18.67 for females; CYP19A1: short ≤ 8.22 for males and females). Genotypes were S/S (short-short), L/L (long-long), and S/L (short-long) for ERβ and CYP19A1 polymorphisms, and S *vs* L for AR polymorphism. A backward stepwise cross-interaction analysis was performed, starting with the most complex model (combined effect of all polymorphisms) and continuing to the simplest one (pairwise effects). False positive rate in these multiple tests was controlled with the Bonferroni correction, adjusting the significance cutoff to the number of tests conducted in each step.

In all cases, a missing value for any response, polymorphism, or covariate was cause for exclusion of that individual from the analysis, considering a *p*-value lower than 0.05 as significant.

3. Results

3.1. Analyses of tandem polymorphisms

The allele distribution for each tandem polymorphism was analyzed within two independent populations taking into account the biological sex: MtF *vs* male controls and FtM *vs* female controls. A total of 19 different alleles were identified for the ERβ (ranging from 18 to 41 repeats), 23 for AR (ranging from 6 to 33) and 12 for CYP19A1 (ranging from 4 to 24), see Supplemental Figure S2) There were not significant differences between MtFs and control males in the distribution of repeat numbers of ERβ (U = 358850; Z = −0.49; p = 0.623), AR (Mann-Whitney U = 88953.5; Z = −0.56, p = 0.578), and CYP19A1 (U = 359230; Z = −1.83; p = 0.067). Between FtMs *vs* female controls, only the ERβ polymorphism showed significant differences (U = 205830; Z = −2.90; p = 0.004), in contrast to AR (Mann-Whitney U = 217790.5; Z = 1.18; p = 0.236), and CYP19A1 (U = 230290.5; Z = −0.81; p = 0.418).

3.2. Analyses of single nucleotide polymorphisms

The allele and genotype frequencies for the XbaI-ERα polymorphism were significantly different between FtMs and female controls ($\chi2$ = 4.049; p = 0.044; and $\chi2$ = 11.237; p = 0.004, respectively). In MtFs *vs* male controls, neither the allele nor the genotype frequencies were significantly different ($\chi2$ = 2.686; p = 0.101; and $\chi2$ = 5.366; p = 0.068, respectively) (Table 1).

Table 1
XbaI-ERα allele/genotype frequencies in male-to-female *vs* control XY and female-to-male *vs* control XX groups.

Allele/Genotype frequencies	All subjects	Control XY	MtF	$\chi2$	*P*
A	0.625	0.672	0.599	2.686	0.101
G	0.375	0.328	0.401		
A/A	0.394	0.484	0.343	5.366	0.068
A/G	0.463	0.376	0.512		
G/G	0.143	0.140	0.145		
Allele/Genotype frequencies	**All subjects**	**Control XX**	**FtM**	$\chi2$	*P*
A	0.633	0.584	0.675	4.049	0.044*
G	0.367	0.416	0.325		
A/A	0.404	0.299	0.496	11.237	0.004*
A/G	0.457	0.570	0.358		
G/G	0.139	0.131	0.146		

MtF: male-to-female transsexuals; **FtM**: female-to-male transsexuals.

3.3. Cross interaction analysis

For the cross interaction analyses, the tandem polymorphisms were dichotomized into short and long allele groups. The cross interaction analyses were performed stepwise starting with the most complex model (ERβ with CYP19A1 and XbaI-ERα polymorphisms by AR), and then pairwise comparisons (ERβ with CYP19A1 and ERβ with XbaI-ERα by AR), always considering somatically females and somatically males as two independent populations.

3.4. Interaction analysis between polymorphisms ERβ, CYP19A1 and XbaI-ERα by AR

The cross interaction between ERβ, CYP19A1 and XbaI-ERα adjusted by AR showed statistical significance in the somatically males (Supplemental Table 2) and in the somatically females (Supplemental Table 3). The OR for transsexuality was greater for somatically males carrying a short allele for ERβ, a long allele for aromatase, a G allele for XbaI-ERα and a short allele for AR (SLGS) [OR = 15.74 (1.14–218.14); p = 0.0394], using the SSAS genotype as reference category (short allele for ERβ, short allele for aromatase, A allele for XbaI-ERα and short allele for AR). For somatically females carrying a long allele for ERβ, a short allele for aromatase, an A allele for XbaI-ERα and a genotype S/S for AR (LSA, A/A) [OR = 16.91 (1.37–208.58); p = 0.0271], using the SSA A/A genotype as reference category (Supplemental Table 4). However, the differences were not significant when Bonferroni corrections were used. The interaction between ERβ, CYP19A1 and XbaI-ERα by AR for susceptibility to transsexualism in somatically males and somatically females was significant (p = 0.0038 and p = 0.0029 respectively).

3.5. Interaction analysis between polymorphisms ERβ and CYP19A1 by AR

The cross interaction between ERβ and CYP19A1 adjusted by AR showed statistical significance in somatically males (Table 2) and in somatically females (Supplemental Table 5). The OR was significantly lower for MtFs carrying the LLL genotype: long allele for ERβ, long allele for CYP19A1 and long allele for AR [OR = 0.52 (0.35–0.79); p = 0.0016], remaining significant after Bonferroni correction (0.05/7 = 0.007), compared to the reference category SSL genotype: short allele for ERβ, short allele for CYP19A1 and long allele for AR. The OR was significantly higher for FtMs carrying the LLS/L genotype: long allele for ERβ, long allele for CYP19A1 and S/L genotype for AR

Table 2
Logistic regression analysis results for possible cross interaction between ERβ and CYP19A1 by AR, and OR for MtF transsexualism.

ERβ and CYP19A1 by AR cross-classification interaction table (n = 855, crude analysis)						
			AR			
Alleles			L		S	
ERβ	CYP19A1	Frequency	OR (95% CI)	*P*	OR (95% CI)	*P*
S	S	0.2691	**1.00 (reference)**		0.33 (0.14 - 0.77)	0.0107*
L	L	0.2665	0.52 (0.35 - 0.79)	0.0016*†	0.47 (0.21 - 1.03)	0.0623
L	S	0.2244	0.65 (0.37 - 1.12)	0.1274	0.46 (0.22 - 0.96)	0.0384*
S	L	0.2400	0.77 (0.47 - 1.25)	0.2989	0.37 (0.18 - 0.77)	0.0073*

Interaction *p*-value: 0.0076*.
The risk for each genotype is compared with regard to the reference category **1.00 (reference):** SSL: short allele for ERβ, short allele for aromatase, and long allele for AR. *Statistically significant ($p \leq 0.05$). †Significant after the Bonferroni correction ($p < 0.05/7 = 0.007$).

[OR = 3.33 (1.11–10.02); $p = 0.0318$], but the differences were not significant when Bonferroni corrections were used (0.05/11 = 0.0045). The interaction between ERβ and CYP19A1 by AR was significant for susceptibility to transsexualism in somatically males and somatically females ($p = 0.0076$ and $p = 0.0012$ respectively).

3.6. Interaction analysis between polymorphisms ERβ and XbaI-ERα by AR

The interaction between ERβ and XbaI-ERα by AR was only significant in somatically males ($p = 0.001$) (Table 3), not in somatically females (Supplemental Table 5). The cross interactions between ERβ and XbaI-ERα adjusted by AR showed that in somatically males the OR was significantly lower carrying a short allele for ERβ, an A allele for XbaI-ERα and a short allele for AR [OR = 0.10 (0.03 - 0.39; $p = 0.0004$], remaining significant after Bonferroni correction (0.05/7 = 0.007) (Table 3).

3.7. Interaction analysis between the polymorphisms by AR

The analyses of the polymorphisms by AR only showed significant differences in ERβ in somatically males (Tables 4 and 5) with interaction *p*-values of 0.001 and 0.0034. Somatically males carrying the long AR allele with genotype S/L for ERβ [OR = 0.40 (0.23 - 0.70); $p = 0.0013$] (Table 4) was a combination with statistical significance that remained significant after Bonferroni correction. There is also an inverse relationship between the long AR allele in combination with the ERβ S/S genotype (Table 5) [OR = 2.81 (1.38–5.70); $p = 0.0043$] that increase the risk of transsexuality that remained significant after Bonferroni correction (Table 5).

Table 4
Binary logistic regression analysis of ERβ by AR in male-to-female and control XY groups, and OR for MtF transsexualism.

ERβ within AR (n = 827, crude analysis)					
AR	ERβ	Control XY	MtF	OR (95% CI)	*P*
L	S/S	0.05	0.12	**(1.00 reference)**	
	S/L	0.34	0.30	0.40 (0.23 – 0.70)	0.0013*†
	L/L	0.12	0.11	0.41 (0.22 – 0.78)	0.0057*†
S	S/S	0.11	0.12	**(1.00 reference)**	
	S/L	0.35	0.27	0.72 (0.45 - 1.16)	0.1747
	L/L	0.04	0.10	2.36 (1.16 - 4.78)	0.0173*

Interaction *p*-value = 0.001*.
MtF: male-to-female transsexuals. The risk for each genotype is compared with regard to the reference category **1.00 (reference):** S/S genotype for ERβ. *Statistically significant ($p \leq 0.05$). †Significant after the Bonferroni correction ($p < 0.05/4 = 0.0125$).

4. Discussion

Our study resulted in three main findings. First, there is an interaction between the ERβ and AR polymorphisms in the development of atypical gender identity in the MtF population involving an inverse relationship between these polymorphisms. Second, the development of gender in the FtM population is associated with ERβ and/or ERα, but no interaction between these polymorphisms was found. Third, both ERs (α and β) are involved in typical male and female gender development.

The androphilic MtF population presents an inverse relationship between ERβ and AR such that the short AR polymorphism is associated with the L/L ERβ genotype, while, on the contrary, the long AR polymorphism is associated with the S/S ERβ genotype.

Neither of these two polymorphisms on its own is associated with

Table 3
Logistic regression analysis results for possible cross interaction between ERβ and XbaI-ERα by AR, and OR for MtF transsexualism.

ERβ and XbaI-ERα by AR cross-classification interaction table (n = 884, crude analysis)						
			AR			
Alleles			L		S	
ERβ	XbaI-ERα	Frequency	OR (95% CI)	*P*	OR (95% CI)	*P*
S	A	0.3668	**1.00 (reference)**		0.10 (0.03 - 0.39)	0.0004*†
L	A	0.2964	0.56 (0.28 - 1.10)	0.0964	0.37 (0.15 - 0.91)	0.0303*
L	G	0.1950	0.82 (0.45 - 1.50)	0.5289	0.25 (0.08 - 0.79)	0.0175*
S	G	0.1418	0.90 (0.28 - 2.88)	0.8692	1.22 (0.24 - 6.16)	0.8218

Interaction *p*-value = 0.001*.
The risk for each genotype is compared with regard to the reference category **(1.00 reference).** *Statistically significant ($p \leq 0.05$). †Significant after the Bonferroni correction ($p < 0.05/7 = 0.007$).

Table 5
Binary logistic regression analysis of AR within ERβ in male-to-female and control XY groups, and OR for MtF transsexualism.

AR within ERβ (n = 827, crude analysis)					
ERβ	AR	Control XY	MtF	OR (95% CI)	*P*
S/S	**L**	24	44	**(1.00 reference)**	
	S	49	44	0.49 (0.26-0.93)	0.0279*
S/L	**L**	151	112	**(1.00 reference)**	
	S	156	101	0.87 (0.62-1.24)	0.4392
L/L	**L**	53	40	**(1.00 reference)**	
	S	17	36	2.81 (1.38-5.70)	0.0043*†

Test for interaction in the trend = 0.0034*.
MtF: male-to-female transsexuals. The risk for each genotype is compared with regard to the reference category **1.00 (reference)**. *Statistically significant ($p \leq 0.05$). †Significant after the Bonferroni correction ($p < 0.05/3 = 0.0166$).

MtF. AR is necessary, but insufficient on its own without ERβ for gender development in MtF. The OR for the interaction between ERβ and AR is heightened by a further association with the XbaI-ERα polymorphism. The highest risk for transsexuality is observed in somatically male individuals carrying a short allele (S) for the ERβ polymorphism together with a G allele for XbaI-ERα and a short allele (S) for AR (SGS genotype) compared to the reference category SAS, short allele (S) for the ERβ together with an A allele for XbaI-ERα and a short allele (S) for AR. However, the differences were not significant when Bonferroni corrections were used

Furthermore, there is a lower risk for transsexuality in somatically male individuals when the short allele (S) for AR is associated with the short allele (S) for ERβ and the A allele for ERα (SAS genotype) compared to the reference category SAL, short allele (S) for the ERβ together with an A allele for XbaI-ERα and a long allele (L) for AR.

Previous studies evaluated polymorphism interactions using a binary logistic regression model (Henningsson et al., 2005; Hare et al., 2009; Ujike et al., 2009). However, cross-interaction analysis between polymorphisms is additionally used here. Our results confirmed those obtained by Henningsson et al. (Henningsson et al., 2005), who suggested an interaction between ERβ and AR, but, what is more, we are able to specify the genotypes involved. We found that fewer CAG repeats in the AR polymorphism increases the risk of transsexuality in comparison to the presence of a higher number of CAG repeats, in interaction with the L/L genotype for ERβ (Table 4). Like Hare et al. (2009), we also found an association between the AR polymorphism and MtF. However, we found, the association was restrictive since a low number of CAG repeats in the AR increases the risk of transsexuality in interaction with the L/L genotype for ERβ (Table 4), and, *vice versa,* more CAG repeats in the AR increases the risk of transsexuality in interaction with the S/S genotype for the ERβ (Table 5). The Ujike et al. study (Ujike et al., 2009) is not really comparable to ours or other studies mentioned above because it used the average instead of the median to establish long and short alleles. Considering the work of Henningsson et al. (2005) and Hare et al. (2009) together with our results, and taking into account the different origins of the analyzed populations, we could say that the implication of the AR in gender dysphoria in MtF is a consistent finding.

ER α and β also play a key role in the gynephilic FtM population. Specific variants of ERβ and ERα polymorphisms are associated with FtM. Interestingly, there is no interaction between these polymorphisms. ERα, particularly the XbaI-ERα polymorphism, has a significant effect: an A/A genotype implied a greater susceptibility to transsexuality, while genotype A/G showed a protective effect. With respect to the ERβ polymorphism, we found a direct association between the number of CA repeats and transsexuality, confirming our previous report (Fernández et al., 2014a).

One important observation that is directly derived from our analysis is that androphilic MtFs and gynephilic FtMs share a common feature: the involvement of the same polymorphisms in the estrogen receptors. Moreover, these polymorphisms have been related to sexually dimorphic behavior like Alzheimer's disease, depression, obsessive compulsive disorder, schizophrenia, FtM dysphoria and others (Brandi et al., 1999; Ji et al., 2000; Corbo et al., 2006; Boada et al., 2012; Pan et al., 2014).

Estrogen is an important regulator of brain growth and differentiation and the ERs have a key function in sexual differentiation of brain and behavior (McCarthy, 2008). Additionally, ER α and β are found in both the developing (González et al., 2007) and adult human brain (Osterlund et al., 2000). ER expression shows sex differences (Ishunina et al., 2002).

With respect to the typical masculinization of the brain in XY subjects, it was proposed that direct androgen action on the brain is crucial for the development of a male gender identity and heterosexuality and that the aromatization theory, developed from rodent experiments, would be of secondary importance in our species (Swaab, 2004). In contrast, our results show that both ERs and AR receptors are involved in the development of transsexuality in the androphilic MtF population. As well as by androgens acting on AR, ERs can be activated by estradiol resulting from the aromatization of testosterone (Lephart, 1996). The aromatase enzyme is already present in human fetuses (Naftolin et al., 1971). Moreover, dihydrotestosterone, a reduced testosterone metabolite, can be further metabolized to 5α-androstene-3β,17β-diol, a molecule that preferentially binds to ERβ (Kuiper et al., 1997). Our results show the involvement of ERα and β in the typical development of gender in men and women.

At the present stage, it is very difficult to fit molecular, brain and behavioral findings together. This is because there are complex interactions among hormones, receptors and enzymes (Swift-Gallant and Monks, 2017), as well as species-specific behaviors, and the expression of sex differences in the brain shows two morphological patterns under different hormonal controls (Guillamón and Segovia, 1996). MRI studies before cross-sex hormone treatment show that androphilic MtFs and gynephilic FtMs present a type of intersexuality restricted to the brain. The brain of androphilic MtFs shows a mixture of masculine, feminine and demasculinized traits, and gynephilic FtMs also show a mixture of feminine, masculine and defeminized traits (Guillamón et al., 2016). Both groups share common genotypic and phenotypic features: first, the involvement of the two ERs in gender dysphoria, and second, their cortex is thicker than gender-typical males, but this happens in different regions than in males (Guillamón et al., 2016). These observations support our hypothesis that MtFs and FtMs undergo an atypical developmental process with respect to the sexual differentiation of their cortex (Guillamón et al., 2016) (Fig. 1).

5. Conclusions

We have found that key receptors implicated in sexual differentiation of the brain have a specific allele combination for ERβ, ERα, and AR in the MtF population, whose gender differentiation is associated with a specific genotypic combination of ERs and AR polymorphisms. Also, FtM gender is associated with specific polymorphisms of the ERβ and ERα receptors. Thus, ERα and ERβ play a key role in the typical sexual differentiation of the brain in our species.

Declarations of interest

None.

Contributors

Each author declares his/her individual contribution to the article. All authors have participated in the research and/or article preparation. All authors have approved the final article.

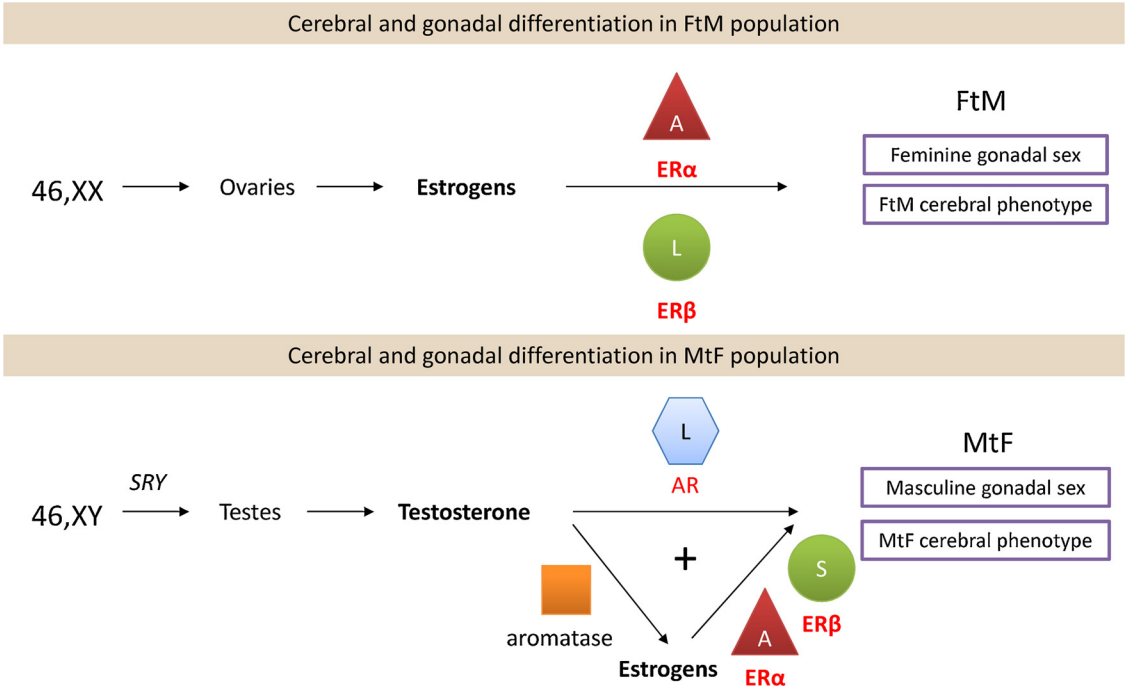

Fig. 1. Hypothetical development of gender dysphoria in gynephilic FtM and androphilic MtF populations during prenatal period. Estrogens could act directly *via* ERα and/or ERβ in the FtM population. In MtFs, but not in FtMs, the intervention of testosterone *via* AR is also necessary. Specific variants (alleles) could affect the level of ERα and ERβ expression. S = Short allele; L = Long allele; A = Adenine.

Authorship

All authors have made substantial contributions to all of the following:

(1) The conception and design of the study (RF, AG, EP), diagnosis (EGG, IE, MCA, MM, GA) and acquisition of data (RF, JCC, EGG, IE, MCA, MM, GA, EP), or analysis and interpretation of data (RF, AG, AJ, EP).

(2) Drafting the article or revising it critically for important intellectual content (RF, AG, EP).

(3) All authors have approved the final article.

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Conflicts of interest

The authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.07.032.

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