Association Study of *ER*β, *AR*, and *CYP19A1* Genes and MtF Transsexualism

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ABSTRACT

Introduction. The etiology of male-to-female (MtF) transsexualism is unknown. Both genetic and neurological factors may play an important role.

Aim. To investigate the possible influence of the genetic factor on the etiology of MtF transsexualism.

Methods. We carried out a cytogenetic and molecular analysis in 442 MtFs and 473 healthy, age- and geographical origin-matched XY control males. The karyotype was investigated by G-banding and by high-density array in the transsexual group. The molecular analysis involved three tandem variable regions of genes estrogen receptor β (*ER*β) (CA tandem repeats in intron 5), androgen receptor (*AR*) (CAG tandem repeats in exon 1), and *CYP19A1* (TTTA tandem repeats in intron 4). The allele and genotype frequencies, after division into short and long alleles, were obtained.

Main Outcome Measures. We investigated the association between genotype and transsexualism by performing a molecular analysis of three variable regions of genes ERβ, *AR*, and *CYP19A1* in 915 individuals (442 MtFs and 473 control males).

Results. Most MtFs showed an unremarkable 46,XY karyotype (97.96%). No specific chromosome aberration was associated with MtF transsexualism, and prevalence of aneuploidy (2.04%) was slightly higher than in the general population. Molecular analyses showed no significant difference in allelic or genotypic distribution of the genes examined between MtFs and controls. Moreover, molecular findings presented no evidence of an association between the sex hormone-related genes (*ER*β, *AR*, and *CYP19A1*) and MtF transsexualism.

Conclusions. The study suggests that the analysis of karyotype provides limited information in these subjects. Variable regions analyzed from *ER*β, *AR*, and *CYP19A1* are not associated with MtF transsexualism. Nevertheless, this does not exclude other polymorphic regions not analyzed. **Fernández R, Esteva I, Gómez-Gil E, Rumbo T, Almaraz MC, Roda E, Haro-Mora J-J, Guillamón A, and Pásaro E. Association study of *ER*β, *AR*, and *CYP19A1* genes and MtF transsexualism. J Sex Med 2014;11:2986–2994.**

Key Words. Androgen Receptor; Aromatase; Estrogen Receptor; Gender Dysphoria; Male-to-Female Transsexuals; Transsexualism

Introduction

Gender identity disorders (GIDs) are characterized by persistent cross-gender identification and discomfort with the individual's assigned gender (American Psychiatric Association) [1]. The disorders are manifested by cross dressing and a search for hormonal and surgical sex reassignment to the desired anatomical sex. Transsexualism is an extreme form of GID.

It is not possible to identify a single cause for transsexualism. Biological studies have shown that

it is associated with neurodevelopmental processes of the brain [2–6], while others imply the involvement of genetic factors [7–10]. Furthermore, different psychological theories have also been proposed [11].

Sexual differentiation of the brain in mammals is significantly influenced by sex hormones and other circulating hormones [12], such as androgens, estrogens, and enzymes for the conversion of androgens to estrogens. The androgen receptor (*AR*) is implicated in the differentiation of the cortex. The possession of an allele with a smaller number of repeats confers more efficient functioning of the receptor and is associated with "masculinization" of the cortex in adolescence [13].

For the estrogen receptor (*ER*), two subtypes, the alpha *ER*α and beta *ER*β, have been identified [14]. Expression of the beta subtype is clearly higher in several brain regions [15], and male mice lacking functional *ER*β have an incompletely defeminized brain and behavior [16].

Moreover, animal studies have clearly demonstrated that prenatal exposure to testosterone plays a primary role in neural and behavioral sexual differentiation [17]. Testosterone binds to and activates *AR*s and is converted to estrogen by aromatase (*CYP19A1*) in the brain and consequently activates the central *ER*α and *ER*β. It may cause masculinization directly by activation of *AR* or indirectly by activation of *ER*s [18,19]. Aromatase cytochrome P450 (*CYP19A1*), which is necessary for the conversion of androgens to estrogens, plays an important role in the sexual differentiation of the brain. In humans, the gene *CYP19A1* is expressed in multiple areas of the brain, notably the temporal and frontal neocortex, the hippocampus, and the hypothalamus [20,21]. It is believed that sex differences in estrogen levels as a result of aromatization of androgen may explain the sexual dimorphisms found in the hypothalamus [22].

Hence, these three genes *ER*β, *AR*, and *CYP19A1* are strong candidates in the quest for genes that may influence the likelihood of developing gender dysphoria.

Previous studies on these genes show discordant results [7–10]. Henningsson et al. [9], in a genetic study of transsexualism in a population consisting of 29 male-to-female (MtF) transsexuals from Sweden, found significant differences when they examined the *ER*β gene but not with respect to two other studied polymorphisms (*AR* and *CYP19A1*). Hare et al. [7], in a population consisting of 112 MtFs from Australia and Los Angeles (California) and 258 control nontranssexual males from Australia, found a significant association between longer *AR* gene polymorphisms and MtF. Moreover, Ujike et al. [8], in a Japanese population of 168 FtMs and 74 MtFs, found no significant differences in allelic or genotypic distributions of any gene examined (*AR*, *ER*α, *ER*β, *CYP19A1*, and six polymorphisms: rs2008112, rs508653, V660L, H770H, rs572698, and PROGINS) between MtFs and control males or between FtMs and control females. Finally, our team previously analyzed the same genes in 273 FtMs and found that there is an association between *ER*β and FtM. 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 [10].

The aim of our study was to investigate the possible association of the karyotype and the sex hormone-related genes *ER*β, *AR*, and *CYP19A1* with MtF transsexualism by performing a molecular analysis of the variable regions of these genes in 442 MtFs and 473 control males.

Methods

Subjects

The subjects comprised 442 MtFs and 473 age and geographical origin-matched control males. The selection of MtFs was conducted through both the Andalusian Gender Identity Unit (Carlos Haya Hospital of Málaga, Spain) and the Gender Identity Units of Catalonia (Clínic Hospital of Barcelona, Spain).

The diagnoses were made using the *Diagnostic and Statistical Manual of Mental Disorders IV* (American Psychiatric Association) [1] and the International Classification of Diseases, tenth edition [23]. All patients received medical examinations by an endocrinologist to rule out the anomaly of the external genitalia and internal sex organs. Participants had no endocrine, neurological, or major psychiatric comorbidity.

Sociodemographic, clinical, and psychiatric data that included any family background of transsexuality were collected for all patients as part of similar standard clinical assessments at both clinics [24–26]. Subjects included in the sample presented early-onset of gender disphoria and were attracted to subjects of their natal sex. The