

Biol Psychiatry. Author manuscript; available in PMC 2012 July 23

Published in final edited form as:

Biol Psychiatry. 2009 January 1; 65(1): 93–96. doi:10.1016/j.biopsych.2008.08.033.

Androgen Receptor Repeat Length Polymorphism Associated with Male-to-Female Transsexualism

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Abstract

Background—There is a likely genetic component to transsexualism, and genes involved in sex steroidogenesis are good candidates. We explored the specific hypothesis that male-to-female transsexualism is associated with gene variants responsible for undermasculinization and/or feminization. Specifically, we assessed the role of disease-associated repeat length polymorphisms in the androgen receptor (AR), estrogen receptor β ($ER\beta$), and aromatase (CYP19) genes.

Methods—Subject-control analysis included 112 male-to-female transsexuals and 258 non-transsexual males. Associations and interactions were investigated between CAG repeat length in the AR gene, CA repeat length in the $ER\beta$ gene, and TTTA repeat length in the CYP19 gene and male-to-female transsexualism.

Results—A significant association was identified between transsexualism and the AR allele, with transsexuals having longer AR repeat lengths than non-transsexual male control subjects (p = .04). No associations for transsexualism were evident in repeat lengths for CYP19 or $ER\beta$ genes. Individuals were then classified as short or long for each gene polymorphism on the basis of control median polymorphism lengths in order to further elucidate possible combined effects. No interaction associations between the three genes and transsexualism were identified.

Conclusions—This study provides evidence that male gender identity might be partly mediated through the androgen receptor.

Keywords

Androgen receptor; AR; aromatase; CYP19, $ER\beta$; estrogen receptor β ; gender identity disorder; transsexualism

From an early age, people develop an inner sense of being male or female. Transsexuals however, identify with a physical sex opposite to their biological sex. Such individuals might seek to alleviate their distress by altering their bodies through hormone therapy and sex reassignment surgery (1). The prevalence of transsexualism ranges from 1:2,900 to 1:100,000; and little is known about the etiology of this condition (2–4). Some theories have

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suggested that psychosocial factors—including dysfunctional family dynamics (5) and traumatic childhood experiences (6)—lead to the development of a transsexual identity.

Increasingly, biomedical research is implicating biological factors. Co-occurrence among twin pairs, father-son pairs, and brother-sister pairs (7,8) raises the question of whether gender dysphoria is heritable. Anatomical studies show that certain brain structures in maleto-female transsexuals are more "female-like" in volume and neuronal density (9,10). Furthermore, the response to the odor of male and female steroids in male-to-female transsexuals was more similar to that of control women than control men (11). Other studies suggest that sex steroids influence gender identity. Female-to-male transsexuality has been associated with polycystic ovary syndrome and hyperandrogenemia (12). Moreover, female subjects with the disorder of sex development called congenital adrenal hyperplasia are exposed to high levels of androgens prenatally and seem to be at much higher risk of gender identity disorder than the general population (13). A significant association was identified between female-to-male transsexualism and the CYP17 gene (which encodes 17ahydroxylase, the enzyme deficient in some virilized congenital adrenal hyperplasia patients) (14). Aromatase (CYP19), the enzyme that converts testosterone to estrogen, has also been implicated in female gender identity. A 46, XX woman with congenital adrenal hyperplasia carried a null CYP19 mutation, was born with phallic enlargement, a uterus, and ovaries, and exhibited a persistent male gender identity and male gender role behavior (15).

There are few genetic association studies of male-to-female transsexualism. A study of 29 Swedish male-to-female transsexuals identified a significant association with a dinucleotide CA polymorphism in the estrogen receptor β ($ER\beta$) gene (p = .03) (16). It has been suggested that $ER\beta$ has a defeminization role in male brain and behavior, on the basis of knockout mouse studies (17). Altogether, genetic studies on transsexuals suggest that both androgen and estrogen might play a role in gender identity.

We sought to investigate whether sex steroidogenesis genes are associated with male-to-female transsexualism in the largest cohort collected to date. We analyzed the variable polymorphism lengths of three genes—androgen receptor (AR), $ER\beta$, and CYP19—in Caucasian transsexuals and compared these with non-transsexual male control subjects.

Methods and Materials

Participants

One hundred and twelve Caucasian male-to-female transsexuals, pre- and post-operative, were recruited from Monash Medical Centre (MMC), Victoria, Australia (n = 76) and from University of California, Los Angeles (UCLA) (n = 36) as per criteria in the DSM-IV—some of whom had reports of gender dysphoria in childhood. Almost all transsexual individuals were receiving hormone treatment. Two hundred and fifty-eight Caucasian male control subjects were also recruited from MMC. Ethical approvals for this study were obtained from MMC and UCLA, and consent procedures adhered to the tenets of the Declaration of Helsinki. The sexuality is only known for approximately 40% of patients, because some patients did not wish to discuss or disclose this information or the patient's sexuality was flexible and not easily classified.

Genotyping

Genomic DNA was extracted from whole blood (18) or saliva (Oragene T). Androgen receptor exon 1 CAG repeat was amplified with polymerase chain reaction with VIC-labelled 5'-TCTGGAT-CACTTCGCGCAC-3' and 5'-GTTCCTCATCCAGGACCAGGTA-3'. The $ER\beta$ intron 5 CA repeat was amplified with FAM-labelled 5'-GGTACAGACCATGGTTTACC-3', and 5'-AACAAAATGTT-

GAATGAGTGGG-3. The *CYP19* intron 4 TTTA repeat was amplified with NED-labelled 5'-GGTACTTAGCTACAATC-3', and 5'-GGGTGATAGAGTCAGAGCCT-3'. Polymerase chain reaction was 95°C for 30 sec, 30 sec at 59°C for *AR*, 55°C for *ER*β, and 58°C for *CYP19*, and extension at 72°C for 30 sec for 35 cycles. The polymerase chain reaction products from the three genes were then mixed for each individual with Genescan LIZ-500 size standard and analyzed on an ABI Prism 3130*xI* (Applied Biosystems, Foster City, California). Successful genotyping was achieved for at least 101 of the 112 transsexual individuals across the three gene polymorphisms (101 for *AR*, 111 for *ER*β, and 104 for *CYP19*) and 258 control subjects.

Statistics

To evaluate the repeat length polymorphism data for possible associations with male-to-female transsexualism, independent samples t tests were used. Interactions between the three gene polymorphisms were evaluated with a binary logistic regression model. Analyses were performed with Statistical Package for the Social Sciences 12.0 software (SPSS, Chicago, Illinois). A p value < .05 was considered significant. The primary analysis performed was of the association between male-to-female transsexualism and AR, $ER\beta$, and CYP19 genotypes.

Results

Polymorphic fragment lengths for 258 male subjects and 112 transsexuals were obtained. Twenty-one different alleles were identified for the AR gene polymorphism, 14 for the $ER\beta$ gene polymorphism, and 8 for the CYP19 gene polymorphism. The percentages of each allele in the control and transsexual populations are shown in Figure 1. For the AR gene, a difference in the mean repeat length was identified, with transsexuals having significantly longer mean repeat lengths (243.2 base pairs) than control subjects (245.1 base pairs, p = 0.04).

The repeat lengths were then sub-classified to compare genotypes. For the control population, equally sized genotype groups were generated on the basis of the median repeat length for all three genes whereby alleles below this length were assigned as "short" and above this length assigned as "long" (AR short 20 repeats, long > 20 repeats; $ER\beta$: short 22 repeats, long > 22 repeats; and CYP19 short 7 repeats, long > 7 repeats). The number of short and long repeat length alleles for AR, $ER\beta$, and $ER\beta$ are shown in Table 1. The genotypes of $ER\beta$ for all individuals were determined as SS (two short alleles), SL (one short allele and one long), or LL (two long alleles). The $ER\beta$ genotype, being X-linked, is hemizygous, and thus the comparison undertaken was between short and long genotypes. An independent samples $ER\beta$ revealed no significant association for the $ER\beta$ or $ER\beta$ or $ER\beta$ genotypes between the two populations ($ER\beta$). With binary logistic regression analysis we observed no significant interactions for any of the variable combinations for the three genes ($ER\beta$). Table 3).

Discussion

To date, this is the largest genetic study of transsexualism conducted. We observed a significant association between longer AR gene polymorphisms and male-to-female transsexualism. Longer CAG repeats in the AR gene lead to reduced binding of the AR protein to co-activator, due to its inhibitory interaction with the receptor, resulting in less effective testosterone signalling (19), a mechanism typically involved in masculinization of the brain during early development (1). Female subjects typically lack the gonadal testosterone surge that occurs in male subjects. Consequently, the AR gene is not activated

(20). It is possible that a decrease in testosterone levels in the brain during development might result in incomplete masculinization of the brain in male-to-female transsexuals, resulting in a more feminized brain and a female gender identity.

A recent study on female-to-male transsexuals identified a *CYP17* single nucleotide polymorphism that was significantly associated with the occurrence of transsexualism (14). These individuals have a higher serum testosterone level than control female subjects, the converse effect of what is suggested in our study of male-to-female transsexualism. The effect we identified was weak; thus it seems highly likely that male-to-female transsexualism is due to multiple genetic factors.

We were unable to replicate the significant association between longer CA repeat lengths in the $ER\beta$ gene and male-to-female transsexualism, contrary to previous findings in 29 Swedish male-to-female transsexuals (16), even though we undertook the same statistical analysis that they used. Our sample size was approximately four times larger than that of the Swedish study, so it is possible that the former study was underpowered to detect a false positive. Alternatively, there might be differences between Swedish and non-Swedish populations in this polymorphism. In the Swedish study, the long repeat occurred in 51.8% of control subjects and 67.1% of transsexuals (16), whereas in the present study the long repeat occurred in 36.5% of control subjects and 44.1% of transsexuals. Thus, although there was a trend in the same direction in both studies, there are major differences in prevalence of these long repeats between the two populations.

In conclusion, our findings indicate a significant association between male-to-female transsexualism and the long polymorphism for the *AR* repeat. This finding links the androgen receptor and further implicates genes in the steroidogenesis pathway as playing a role in male-to-female transsexualism. We speculate that reduced androgen and androgen signalling might contribute to the female gender identity of male-to-female transsexuals. Further studies including replication in other populations, larger patient collections, and analysis of other polymorphisms, both for the genes studied here and other sex steroidogenesis genes, should be undertaken.

Acknowledgments

Support was provided by the National Health and Medical Research Council Program Grant 33414 (to VRH) and the National Institutes of Health training Grant 5 T32 HD07228 (to FS). All authors have reported no biomedical financial interests or potential conflicts of interest.

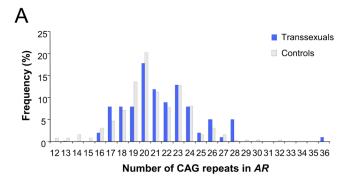
We thank Vivien Vasic for assisting with genotyping, research nurse Elise Forbes for collecting samples, Professor Don Bowden for the donation of control DNAs, and Dr. Izabella Czajka for criticism. We also thank the individuals who voluntarily participated and who are an integral part of this research. This manuscript is dedicated to Dr. Herbert Bower, who inspired us.

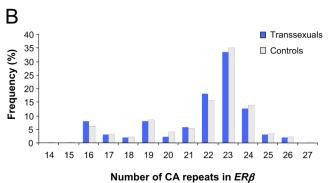
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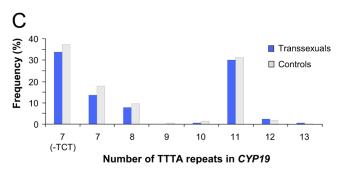


Figure 1. Graphical representation of the allele frequency distributions of (A) androgen receptor (AR), (B) estrogen receptor B (ERB), and (C) aromatase (CYP19) genes showing repeat number for each gene polymorphism. Individuals with 7 TTTA repeats with further classified into those with or without the three base pair TCT insertion polymorphism within this polymorphism.

Table 1
Allele Frequency Numbers for Short and Long Alleles in Both the Transsexual and Control Populations Across all Three Genes

Gene	Polymorphism	Transsexuals n (%)	Control Subjects n (%)
AR	Short	45 (44.6)	135 (52.3)
	Long	56 (55.4)	123 (47.6)
ERβ	Short	106 (47.7)	229 (44.7)
	Long	116 (52.3)	283 (55.3)
CYP19	Short	113 (54.3)	285 (55.2)
	Long	95 (45.7)	231 (44.8)

AR, androgen receptor; ERB, estrogen receptor $\beta;$ CYP19, aromatase.

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Table 2 Genotype Frequency ORs of Transsexualism Among $\textit{ER}\beta$ and CYPI9 Genes

Locus	Repeat	Genotype	Transsexuals $n = 112$ (%)	Locus Repeat Genotype Transsexuals $n = 112$ (%) Control Subjects $n = 258$ (%) OR (95% CI) p	OR (95% CI)	d
ERβ	CA	SS	75 (67.6)	165 (64.0)	1.00 (reference) 77	TT.
		SL	32 (28.8)	83 (32.2)	1.15 (.35–3.79)	.81
		LL	4 (3.6)	10 (3.8)	.96 (.28–3.30)	.95
CYP19	CYP19 TTTA	SS	31 (29.8)	76 (29.5)	1.00 (reference)	.87
		$S\Gamma$	51 (49.0)	133 (51.5)	.91 (.47–1.75)	<i>TT</i> :
		LL	22 (21.2)	49 (19.0)	.45 (.47–1.55)	.60

OR, odds ratio; CI, confidence interval; ERB, estrogen receptor beta; CYP19, aromatase; LL, two long alleles; SL, one short and one long allele; SS, two short alleles.

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Table 3
Logistic Regression Analysis Results for Possible Gene Polymorphism Interactions and Their Association with the Occurrence of Transsexualism

Gene Repeat	p	OR	CI
AR	.38	1.70	(.53-5.48)
CYP19	.93	1.09	(.17-6.94)
ERβ	.97	.98	(.37-2.60)
AR-CYP19	.69	.59	(.04-7.93)
AR-ERβ	.72	.78	(.20-3.02)
ERfj-CYP19	.88	.85	(.11-6.73)
AR-ERQ-CYP19	.87	1.28	(.07-22.43)

Abbreviations as in Tables 1 and 2.