Genetic Link Between Gender Dysphoria and Sex Hormone Signaling

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Context: There is a likely genetic component to gender dysphoria, but association study data have been equivocal.

Objective: We explored the specific hypothesis that gender dysphoria in transgender women is associated with variants in sex hormone–signaling genes responsible for undermasculinization and/or feminization.

Design: Subject-control analysis included 380 transgender women and 344 control male subjects. Associations and interactions were investigated between functional variants in 12 sex hormone–signaling genes and gender dysphoria in transgender women.

Setting: Patients were recruited from the Monash Gender Clinic, Monash Health, Melbourne, Australia, and the University of California, Los Angeles.

Patients: Caucasian (non-Latino) transgender women were recruited who received a diagnosis of transsexualism [Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV) or gender dysphoria (DSM-V)] pre- or postoperatively. Most were receiving hormone treatment at the time of recruitment.

Main Outcome Measured: Genomic DNA was genotyped for repeat length polymorphisms or single nucleotide polymorphisms.

Results: A significant association was identified between gender dysphoria and *ERα*, *SRD5A2*, and *STS* alleles, as well as *ERα* and *SULT2A1* genotypes. Several allele combinations were also overrepresented in transgender women, most involving *AR* (namely, *AR-ERβ*, *AR-PGR*, *AR-COMT*, *CYP17-SRD5A2*). Overrepresented alleles and genotypes are proposed to undermasculinize/feminize on the basis of their reported effects in other disease contexts.

Conclusion: Gender dysphoria may have an oligogenic component, with several genes involved in sex hormone–signaling contributing. **(*J Clin Endocrinol Metab* 104: 390–396, 2019)**

Gender identity—our sense of being male or female—develops early in life. By age 2 years, most children are able to identify their own gender, which is typically consistent with the sex they were at birth (1, 2). Yet, a small percentage of people will report substantial clinical distress because their sex at birth does not reflect their gender identity (3). In extreme cases, patients will be given the diagnosis of gender dysphoria and may undergo

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Abbreviations: *AR*, androgen receptor; *ERα*, estrogen receptor α; *ERβ*, estrogen receptor β; SNP, single nucleotide polymorphism; UCLA, University of California, Los Angeles; UTR, untranslated region.

medical treatments to better align their anatomy and physiology with their gender identity.

Many identity labels may be used among this group of people, with transgender being a broad, encompassing term for many subtypes. In this report, we focus on transgender women, or people whose sex was male at birth and who later transitioned to be female (historically referred to as male-to-female transsexual). Unlike other people who may be transgender, transgender women take steps to affirm their gender identity by social and/or physical transitioning from their birth sex to their experienced gender through cross-hormone treatment and surgery (4).

The etiology of gender dysphoria is unknown, yet the reported prevalence has been increasing, with most estimates suggesting that as many as 521 in 100,000 males and 265 in 100,000 females experience gender dysphoria (5). Early research into gender dysphoria focused on the belief that it was a psychological condition and suggested that dysfunctional family dynamics (6) and traumatic childhood experiences (7) may contribute to gender dysphoria. However, recent studies point toward a biological basis involving endocrine, neurobiological and genetic factors. For instance, an increased prevalence of gender dysphoria was observed among people who experienced atypical prenatal androgen exposure *in utero*, such as females with congenital adrenal hyperplasia (8–15). Neuroimaging studies revealed specific regions in the brains of transgender women that may be more similar to the brains of women serving as control subjects (than that of men serving as control subjects (16–18). Heritability studies suggest a genetic component: 23% to 33% of monozygotic twin pairs are concordant for gender dysphoria (19).

Candidate gene association studies have begun to investigate whether functional variants in sex hormone–signaling genes are associated with gender dysphoria. It is proposed that functional variants may alter sex hormone signaling, causing atypical sexual differentiation of the developing brains of those who will later experience gender dysphoria (20). Some associations have been identified, including an overrepresentation of long CAG repeats in the *AR* of transgender women (21) and an overrepresentation of the *CYP17* T/C SNP (22, 23), *ERβ* CA repeat (24), and *ERα Xbal* A/G single nucleotide polymorphism (SNP) (25) in transgender men. Other studies found no associations (26–28). Most studies have been limited by small sample sizes and there is a need to reproduce findings in large, independent cohorts.

We hypothesized that gender dysphoria in transgender women is associated with genetic variants in sex hormone–signaling genes responsible for undermasculinization and/or feminization of the brain. The aim of our study was to conduct a genetic association study of 12 sex signaling genes, including *COMT, CYP11A1, HSD17B6, STS*, and *SULT2A1*, which, to our knowledge, have not previously been studied in the context of gender dysphoria. We determined the allele and genotype frequencies of variable polymorphic lengths of seven genes and SNPs of five genes in Caucasian (non-Latino) transgender women and compared these with Caucasian (non-Latino) male control subjects.

Methods and Materials

Participants

A total of 380 Caucasian (non-Latino) transgender women were recruited who received a diagnosis of transsexualism (according to the Diagnostic and Statistical Manual of Mental Disorders-IV) or gender dysphoria [Diagnostic and Statistical Manual of Mental Disorders-V (2)] pre- or postoperatively. The transgender women were recruited from the Monash Medical Centre (MMC), Victoria, Australia (n = 222), and the University of California, Los Angeles (UCLA; n = 158). Most were receiving hormone treatment at the time of recruitment. Also recruited were 344 white male control subjects without gender dysphoria. They also were recruited from Monash Medical Centre (n = 281) and UCLA (n = 63). All transgender women and nontransgender male control subjects were determined to be Caucasian on the basis of their surname if ethnicity was not specified (29). Study subjects who identified themselves as nonwhite were excluded. Ethics approval for this study was obtained from Monash Medical Centre and UCLA, and consent procedures adhered to the tenets of the Declaration of Helsinki.

Genotyping

Genomic DNA was extracted from whole blood (30) or saliva (Oragene; DNA Genotek) samples. Androgen receptor (*AR*) exon 1 CAG repeat; estrogen receptor α (*ERα*) promoter region TA repeat; estrogen receptor β (*ERβ*) intron 5 CA repeat; cytochrome P450 family 11 subfamily A member 1 *(CYP11A1)* promoter region TTTTTA repeat; aromatase (*CYP19*) intron 5 TTTA repeat; and progesterone receptor (*PGR*) CA repeat fragment lengths were amplified by PCR and sized by automated capillary electrophoresis (21). Steroid 5-α reductase 2 (*SRD5A2*) 3′untranslated region (UTR) TA repeat was genotyped by PCR directly followed by gel electrophoresis (31). SNPs in catechol-O-methyltransferase (*COMT*) (exon 3 G/A SNP), cytochrome P450 17α-hydroxylase/17,20-lyase (*CYP17*) (5′UTR T/C SNP), 17β hydroxysteroid dehydrogenase 6 (intron 1 T/C SNP), steroid sulfatase (*STS*) (intron 2 A/G SNP), and sulfotransferase (*SULT2A1*) (3′UTR T/C SNP) were determined by agarose gel electrophoresis, after PCR and restriction enzyme digestion (31–34). Oligonucleotide pairs, restriction enzymes, and optimal annealing temperatures used are shown in Supplemental Table 1. Genotyping data were removed if the study subject was determined to be nonwhite or, postgenotyping, to have a disorder of sex development, or if a sample had a genotype failure rate >20%. The removed samples are shown in Supplemental Table 2. Each gene was successfully genotyped for the variants described previously, with haploid chromosome numbers determined for 320 to 343 transgender

women and 259 to 283 nontransgender male control subjects (>90% of the sample cohort).

Statistics

To evaluate the repeat-length polymorphism data for possible associations with transgender women, the distribution of repeat lengths of *AR, ERα*, *ERβ*, *CYP11A1*, *CYP19*, and *PGR* were analyzed using the nonparametric Mann-Whitney *U* test. Repeat lengths were divided into short or long alleles on the basis of the median repeat length of the male control group. Allele and genotype frequencies of all 12 genes (stratified repeat-length polymorphisms and SNPs) were compared using the χ^2 test for independence. Binary logistic regression was used to measure of the strength of the associations with gender dysphoria and evaluate possible interactions among the 12 genes. Analyses were performed using SPSS, version 23.0 (IBM). A p value < 0.05 was considered significant.

Results

Polymorphic fragments lengths were obtained for 320 to 343 transgender women and 269 to 283 male control subjects. The number of repeats identified for each allele is shown in Supplemental Table 3. Median values were calculated from 24 repeat-length distributions. A difference in median repeat length was identified, with transgender women having a significantly shorter median repeat length (16 TA repeats) for *ERα* when compared with male control subjects (17 TA repeats; $P = 0.03$; Table 1).

Allele frequencies were determined for the five SNPs *COMT, CYP17, HSD17B6, SULT2A,1* and *STS*, and the dichotomous *SRD5A2* repeat-length polymorphism (Table 2). A difference between transgender women's and male control subjects' allele frequencies was identified in two of the six genes. Specifically, in transgender women compared with male control subjects, there was an overrepresentation of the TA (9 repeats) repeat in *SRD5A2* (12.3% compared with 8.5%, respectively; $P = 0.030$) and the G allele in *STS* (91.2% compared with 85.9%, respectively; $P = 0.015$).

To compare genotypes, repeat lengths were assigned to "short" or "long" allele groups on the basis of the median repeat length of the control population for the six repeat-length polymorphism genes (*AR*, short ≤22, long >22; *CYP11A1*, short ≤4, long >4; *CYP19*, short ≤7, long >7; *ERα*, short, ≤17, long >17; *ERβ*, short ≤23, long >23; and *PGR*, short ≤18, long >18). The genotypes of *CYP11A1*, *CYP19, ERα*, *ERβ*, and *PGR* polymorphisms for all individuals were determined as SS (*i.e.*, two short alleles), SL (*i.e.*, one short and one long allele), or LL (*i.e.*, two long alleles). Because the *AR* gene is located on the X chromosome, it is hemizygous and the allele and genotype frequencies are equivalent.

Genotype frequencies of the stratified repeat-length polymorphisms (in *CYP11A1*, *CYP19, ERα*, *ERβ*, *PGR*, and *SRD5A2)* and of the SNPs (in *COMT*, *CYP17*, *HSD17B6*, *STS*, and *SULT2A1*) were analyzed using binary logistic regression. An association was identified between transgender women and the *SULT2A1* homozygous TC genotype ($P = 0.009$) and the *ERα* SS genotype ($P = 0.035$) when compared with male control subjects (Table 3). The ORs indicate that within this population, the likelihood of being transgender increases by 1.61 times (95% CI, 1.13 to 2.31) if an individual possesses the *SULT2A1* TC genotype and increases by 1.65 times (95% CI, 1.04 to 2.63) if an individual possesses the *ERα* SS genotype.

Of possible two-locus gene interactions modeled using binary logistic regression, four interactions were overrepresented in transgender women when compared with male control subjects: *AR- ERβ*, *AR-PGR, AR-COMT,* and *CYP17-SRD5A2* (Table 4). Notably, three of the four interactions involve the long CAG repeats of the *AR*.

Table 1. Comparison of Repeat Length Distributions of *AR, ERα, ERβ, CYP11A1, CYP1,9* and *PGR* Repeat Length Polymorphisms

	Male Control Subjects		Transgender Women		
Gene	No.	Median	No.	Median	Mann-Whitney *U* Test *P* Value
AR	289	22	327	22	0.31
ERα	567	17	640	16	0.03[a]
ERβ	580	23	668	23	0.70
CYP11A1	546	4	668	4	0.55
CYP19	580	7	658	7	0.21
PGR	562	18	676	18	0.20

[a]Statistically significant at $P < 0.05$.

Discussion

To our knowledge, this is the largest study to date of gender dysphoria conducted; 12 genes were examined. Variants within *COMT, CYP11A1, HSD17B6, STS*, and *SULT2A1* have not previously been studied in transgender men or women, to our knowledge. In addition, to our knowledge, our study is the only one of three studies to identify an association between TA repeats in *ERα* and gender dysphoria. Our study did not reproduce the independent associations between *ERβ* and gender dysphoria in transgender women reported by Henningsson *et al.* (35) or the association with long CAG repeats in *AR*, previously identified in a subset of our present cohort (21). However, *ERβ* and AR were identified as overrepresented in transgender women when in combination with

Table 2. Allele Frequencies of the *COMT, CYP17, HSD17B6, SRD5A2, STS*, and *SULT2A1* Single Nucleotide or Dichotomous Polymorphisms

		Male Control Subjects		Transgender Women		
Gene	**Allele**	**No.**	**%**	**No.**	**%**	***P* Value[a]**
COMT	G	303	58.7	356	54.1	0.11
	A	213	41.3	302	45.9	
CYP17	T	354	63.7	384	58.9	0.090
	C	202	36.3	268	41.1	
HSD17B6	T	366	65.6	406	60.2	0.053
	C	192	34.3	268	39.8	
SRD5A2	TA(0)	516	91.5	598	87.7	0.030[b]
	TA(9)	48	8.5	84	12.3	
STS	G	238	85.9	302	92.1	0.015[b]
	A	39	14.1	26	7.9	
SULT2A1	T	419	80.9	504	77.5	0.16
	C	99	19.1	146	22.5	

[a]The *P* value was calculated using the χ^2 test.

[b]Statistically significant at $P < 0.05$.

other genes, supporting their involvement in the development of gender dysphoria.

Associations were identified among genetic variants in *ERα*, *SRD5A2, STS*, and *SULT2A1* and this cohort of transgender women. These genetic variants are suspected to be functional, which permits us to examine the predicted functional effects of the specific polymorphism overrepresented in transgender women. In *ERα*, for example, short TA repeats overrepresented in transgender women are also associated with low bone mineral density in women (36). Therefore, we speculate that estrogen signaling is reduced (37). In *SULT2A1,* the heterozygous TC genotype is overrepresented in transgender women. The minor, C allele of *SULT2A1* is associated with elevated sex hormone–binding globulin (38), a glycoprotein that regulates circulatory sex steroid bioavailability and is present within fetal male blood during early gestation (39). In transgender women with the TC SNP, we speculate that fetal sex hormone–binding globulin levels are increased, which may reduce the effects of circulating hormones. Polymorphisms in two genes were overrepresented in transgender women by allele analysis but not by the (more stringent) genotype analysis. First, TA(9 repeats) of *SRD5A2* is associated with reduced prostate cancer risk likely due to reduced DHT (40), suggesting that levels of the potent androgen DHT could be reduced among transgender women. Second, the G allele in *STS* is associated with reduced enzyme levels; this has been noted mostly in studies of ADHD (41), a condition with fivefold increased incidence of gender dysphoria (42), suggesting a possible overlap in etiology.

Four important, two-locus interactions were identified by binary logistic regression modeling: *AR-ERβ*, *AR-PGR*, *AR-COMT*, and *CYP17-SRD5A2*. Of these, three involved long CAG repeats of the *AR*. Although long CAG repeats in *AR* alone may not have an independent effect on the development of gender dysphoria, this *AR* polymorphism may interact with other genes to increase the likelihood of being transgender. This is consistent with a previous finding by Hare *et al.* (21), who identified an increased proportion of long AR repeat lengths within a subset of the population of transgender women in their study. Long CAG repeats reduce *AR* signaling (43–45). Similarly, long repeats in *ERβ* have been associated with decreased *ERβ* signaling (36), potentially reducing the influence of *ERβ* on the defeminization of the male brain (46). In combination, both genotypes appear to have additive effects on the development of gender dysphoria. In contrast, the interaction of *AR* and *COMT* is unclear where the Met^{158} homozygous genotype is known to reduce *COMT* activity (47), affecting estrogen catechol metabolism. Also, the functional effect of the *PGR* polymorphism is unclear in transgender women. Interaction analysis also identified the specific combination of *SRD5A2* and *CYP17* polymorphism, the former associated with reduced levels of DHT (48), whereas the latter is known to increase sex steroid precursor production (49). It seems plausible that together, these polymorphisms may increase the production of precursor steroids and testosterone, but not of DHT, the more potent androgen form.

A limitation of the current study is that patients and control subjects were obtained from two sites, one each in Australia and the United States, and therefore likely represent genetically different populations. Another limitation of this study is the use of Caucasian surnames as a selection criterion. Future and more detailed genetic

Table 3. Genotype Analysis of the COMT, CYP11A1, CYP17, CYP19, ERα, ERβ, HSD17B6, PGR, SRD5A2, and SULT2A1 Polymorphisms

		Male Control Subjects		Transgender Women		
Gene	**Genotype**	**No.**	**%**	**No.**	**%**	***P* Value (OR; 95% CI)[a]**
COMT	GG	85	32.8	100	30.4	0.06 (1.59; 0.98–2.58)
	GA	135	52.1	156	47.4	
	AA	39	15.1	73	22.2	
CYP11A1	SS	89	33.1	124	37.1	0.24 (0.81; 0.57–1.15)
	SL	138	51.3	155	46.4	
	LL	42	15.6	55	16.5	
CYP17	TT	112	40.3	109	33.2	0.097 (1.34; 0.95–1.91)
	TT	130	46.8	170	51.8	
	CC	36	12.9	85	14.9	
CYP19	SS	86	30.3	83	25.2	0.20 (1.36; 0.85–2.16)
	SL	142	50.2	174	52.9	
	LL	55	19.4	72	21.9	
ERα	SS	75	27	103	32.2	0.035[b] (1.65; 1.04–2.63)
	SL	138	49.6	163	50.9	
	LL	65	23.4	54	16.9	
ERβ	SS	186	65.7	216	64.7	0.81 (1.04; 0.74–1.45)
	SL	85	30	103	30.8	
	LL	12	4.2	15	4.5	
HSD17B6	TT	122	43.7	123	36.5	0.091 (1.53; 0.93–2.51)
	TC	122	43.7	160	47.5	
	CC	35	12.5	54	16	
PGR	SS	81	29.3	106	31.4	0.35 (0.80; 0.50–1.27)
	SL	135	48.9	169	50	
	LL	60	21.7	63	18.6	
SRD5A2	TA(0)/(0)	238	84.4	269	78.4	0.12 (1.42; 0.92–2.18)
	TA(0)/(9)	40	14.2	64	18.7	
	TA (9)/(9)	4	1.4	10	2.9	
SULT2A1	TT	175	67.6	192	58.9	0.009[b] (1.61; 1.13–2.31)
	TC	69	26.6	122	37.4	
	CC	15	5.8	12	3.7	

Abbreviations: L, long allele; S, short allele.

[a] *P* values, ORs, and 95% CIs were calculated using binary logistic regression. ORs >1 indicate an increased likelihood of being transgender compared with the reference allele. ORs <1 indicate a decreased likelihood of being transgender compared with the reference allele.

[b] Statistically significant at $P < 0.05$.

analyses such as genome-wide association studies, would give insight into the ethnicity of the cohort, obviating the need for selection criteria based upon ethnicity.

In summary, the results of our study of transgender women support the hypothesis that gender dysphoria has a polygenic basis, involving interactions among multiple genes and polymorphisms that may alter the sexual differentiation of the brain *in utero*, contributing to the development of gender dysphoria in transgender women. However, although discordance rates for gender dysphoria suggest that genetics plays a role, it is not the sole determinant of gender identity. Genome-wide association studies, and genome and methylome approaches, especially when coupled with neuroimaging or sex steroid measurements, should be undertaken to better understand how genetic variants contribute to gender dysphoria.

Transgender people continue to be subjected to high rates of gender-based discrimination when seeking medical care, employment, and education (50, 51). Although people's civil rights should not hinge on science to validate their individuality and lived experience,

Table 4. ORs and 95% CIs of Statistically Significant Two-Locus Gene Interactions Determined by Binary Logistic Regression Modeling

		95% CI[b]		
Two-Locus Gene Interaction[a]	**OR[b]**	**Lower**	**Upper**	***P* Value[b]**
AR (L) – *ERβ* (SL)	2.78	1.15	6.70	0.023
AR (L) – *PGR* (LL)	5.68	1.63	17.95	0.006
AR (L) – *COMT* (AA)	3.88	1.2	12.56	0.024
CYP17 (A1A2) – SRD5A2(TA(0)/TA(9)	4.09	1.19	14.03	0.025

[a] The interacting genotypes for each gene are shown in parentheses. Fragment-length allele polymorphisms were analyzed as dichotomous short or long variables.

[b] Computed using binary logistic regression (n = 233 transgender women; n = 195 male control subjects).

determining what biological factors contribute to gender dysphoria may influence public opinion and public policies related to the transgender community. More importantly, such knowledge can be used to improve diagnosis and treatment of transgender people (*e.g.*, differentiating which children with gender dysphoria will persist into adulthood, vs which will remit). Therefore, there is a clinical need to investigate further the genetic and biological basis of gender dysphoria.

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