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Effects of sex steroids on the pattern of methylation and expression of the promoter region of estrogen and androgen receptors in people with gender dysphoria under cross-sex hormone treatment

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ABSTRACT

Cross-sex hormone therapy (CHT) is critical for phenotypical and physiological transition in adults with gender dysphoria (GD). However, the impact of the CHT onto the molecular level/epigenetic regulation has not been comprehensively addressed. We postulate that CHT in GD could drive changes at the androgen receptor (*AR*), estrogen receptor alpha (*ESR1*) and estrogen receptor beta (*ESR2*), affecting their DNA methylation pattern and mRNA expression that may influence in the phenotypical changes associated to CHT.

We carried out a prospective observational study on individuals with a diagnosis of GD. 18 subjects (no previous CHT): 12 female to male (FtoM) and 6 male to female (MtoF). An Epityper Mass array TM method was used to study the DNA methylation and Real-time PCR quantitative reverse transcription PCR (qRT-PCR) was used to quantify the gene expression. The analysis of *AR*, *ESR1* and *ESR2* receptor was performed at baseline, 6 and 12 months after CHT. No differences in DNA methylation of *ESR* were found in MtoF, while DNA methylation was increased in FtoM at 6 and 12 months of CHT. The AR showed a significant increase of methylation in MtoF group after 12 months of estrogenic treatment. Regarding the expression analysis, *AR* expression was significantly decreased in FtoM upon CHT treatment. *AR*, *ESR1* and *ESR2* methylation were correlated with anthropometric, metabolic and hormonal parameters in FtoM and MtoF.

Our results support that CHT is associated to epigenetic changes that might affect the response to treatment with sex steroids.

1. Introduction

Cross-sex hormone therapy (CHT) is critical for phenotypical and psychological transition in adults with gender dysphoria (GD). CHT enables the development of the secondary sex characteristics of the desired sex, which is one of the cornerstones of sex reassignment [1–3].

Differences in the rhythm and acquisition of secondary sexual characters are observed in individuals using the same CHT and dosage [4]. Differences in both desired and non-desired effects induced by sex hormones could be related with the localization and action of sex hormones receptors. However, the impact of the CHT onto the molecular level/epigenetic regulation has not been comprehensively addressed nowadays.

Androgens act via the androgen receptor (*AR*) in multiple target tissues; we find them in gray matter as well as in subcortical structures and CHT are related with changes on brain cortical thickness [5]. At peripheral level, *AR* expression in all vascular cells could justify the effects on the phenotype and on cardiovascular risk as they are believed to increase the adhesion of monocytes to endothelial cells. Moreover, differences of *AR* expression in vascular tissues in relation to gender are described, with higher levels in men [6,7].

Estrogen receptors (*ESR*) are two; the estrogen receptor alpha (*ESR1*) is expressed in the uterus, prostate, pituitary, brain, skeletal muscle and blood cells among others, while the estrogen receptor beta

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Table 1
Anthropometric, metabolic and hormonal changes after 6 and 12 months of CHT in FtoM.

Parameters	0 months	6 months	P value between 0 and 6 months	12 months	P value between 6 and 12 months	P value between 0 and 12 months
BMI (kg/m2)	22.5 ± 4.9	23.7 ± 4.3	**0.007**	23.5 ± 4.4	0.505	0.207
WC (cm)	77.2 ± 12.2	77.8 ± 11.3	0.727	75.5 ± 9.6	0.144	0.461
Glucose (mmol/l)	4.9 ± 0.4	4.9 ± 0.5	0.786	4.9 ± 0.2	0.887	0.675
HOMA-IR	2.3 ± 1.6	2.0 ± 0.8	0.406	1.8 ± 0.7	0.511	0.561
TC (mmol/l)	4.0 ± 0.4	4.2 ± 0.5	0.260	4.2 ± 0.5	0.722	0.337
HDL-c (mmol/l)	1.2 ± 0.2	1.1 ± 0.2	0.051	1.0 ± 0.2	0.359	**0.044**
LDL-c (mmol/l)	2.5 ± 0.4	2.7 ± 0.4	**0.041**	2.7 ± 0.3	0.937	0.180
Hb (g/dl)	12.9 ± 1.1	14.4 ± 1.3	< **0.001**	15.0 ± 0.6	0.161	< **0.001**
Hto (%)	39.8 ± 2.9	43.7 ± 3.5	< **0.001**	46.2 ± 1.3	**0.043**	< **0.001**
Leucocytes (mm3)	7747.5 ± 2051.6	9080.0 ± 2614.0	**0.004**	8418.1 ± 2201.5	0.130	0.432
us-CRP (mg/dl)	0.04 ± 0.03	0.11 ± 0.05	**0.004**	0.18 ± 0.28	0.425	**0.012**
LH (UI/l)	7.8 ± 5.0	7.7 ± 8.9	0.969	3.9 ± 3.2	0.216	**0.026**
FSH (UI/l)	7.3 ± 5.5	7.1 ± 5.8	0.882	7.1 ± 8.0	0.841	0.905
Estradiol (pg/ml)	66.7 ± 53.1	133.2 ± 154.7	0.214	46.3 ± 15.1	0.125	0.269
TT (ng/dl)	27.9 ± 12.9	866.0 ± 948.9	**0.010**	781.6 ± 292.9	0.654	< **0.001**
SHBG (nmol/l)	62.7 ± 36.4	33.1 ± 16.9	**0.001**	34.2 ± 21.9	0.881	**0.001**
Androstenedione (ng/dl)	242.2 ± 85.0	451.2 ± 273.2	0.072	275.8 ± 103.1	0.424	**0.043**
DHEAS (µg/ml)	2.3 ± 0.8	2.8 ± 1.2	0.102	2.1 ± 1.7	0.590	0.234
Prolactin (ng/dl)	14.8 ± 5.4	14.9 ± 7.3	0.953	10.6 ± 4.8	0.165	**0.041**

BMI: Body mass index; WC: waist circumference; HOMA-IR: Homeostasis model assessment-insulin resistance; TC: Total cholesterol; HDL-c: high density lipoprotein-cholesterol; LDL-c: low density lipoprotein-cholesterol; Hb: hemoglobin; Hto: Hematocrit; us-CRP: ultrasensitive C-reactive protein; LH: Luteinizing Hormone; FSH: Follicle stimulating hormone; TT: Total Testosterone; SHBG: Sex hormone binding globulin.

Table 2
Anthropometric, metabolic and hormonal changes after 6 and 12 months of CHT in MtoF.

Parameters	0 months	6 months	P value between 0 and 6 months	12 months	P value between 6 and 12 months	P value between 0 and 12 months
BMI (kg/m2)	19.6 ± 2.2	20.3 ± 2.2	0.103	20.2 ± 2.0	0.947	0.332
WC (cm)	71.0 ± 5.4	70.8 ± 3.9	0.904	69.0 ± 6.3	0.332	**0.040**
Glucose (mmol/l)	5.4 ± 0.5	5.3 ± 0.5	0.598	5.0 ± 0.3	0.639	0.296
HOMA-IR	4.6 ± 4.7	3.6 ± 1.4	0.641	2.6 ± 0.7	0.411	0.386
TC (mmol/l)	3.3 ± 0.8	3.2 ± 1.1	0.583	3.6 ± 1.0	0.464	0.427
HDL-c (mmol/l)	0.9 ± 0.1	1.0 ± 0.2	0.555	1.3 ± 0.2	**0.048**	0.065
LDL-c (mmol/l)	1.9 ± 0.8	1.8 ± 0.8	0.427	2.0 ± 0.7	0.907	0.681
Hb (g/dl)	14.6 ± 1.6	14.0 ± 1.1	0.145	13.1 ± 0.7	**0.005**	**0.045**
Hto (%)	43.0 ± 4.0	39.5 ± 2.2	0.070	38.2 ± 1.9	0.140	**0.046**
Leucocytes (mm3)	7071.7 ± 1433.0	7760,0 ± 1065.6	0.161	6172.0 ± 999.4	**0.024**	0.229
us-CRP (mg/dl)	0.02 ± 0.01	0.61 ± 0.73	0.207	0.02 ± 0.02	0.342	0.718
LH (UI/l)	2.8 ± 1.4	2.7 ± 4.1	0.995	12.4 ± 27.1	**0.039**	**0.049**
FSH (UI/l)	3.4 ± 1.7	2.2 ± 2.6	0.229	18.8 ± 41.4	**0.038**	**0.046**
Estradiol (pg/ml)	28.6 ± 11.7	59.0 ± 68.3	0.323	100.4 ± 108.1	0.124	**0.019**
TT (ng/dl)	530.2 ± 74.3	97.2 ± 158.9	0.013	11.7 ± 4.7	0.298	**0.005**
SHBG (nmol/l)	29.7 ± 10.7	38.8 ± 14.1	0.282	67.8 ± 20.9	0.095	**0.020**
Prolactin (ng/dl)	14.6 ± 7.2	31.2 ± 10.6	0.027	21.5 ± 12.8	0.122	0.461

BMI: Body mass index; WC: waist circumference; HOMA-IR: Homeostasis model assessment-insulin resistance; TC: Total cholesterol; HDL-c: high density lipoprotein-cholesterol; LDL-c: low density lipoprotein-cholesterol; Hb: hemoglobin; Hto: Hematocrit; us-CRP: ultrasensitive C-reactive protein; LH: Luteinizing Hormone; FSH: Follicle stimulating hormone; TT: Total Testosterone; SHBG: Sex hormone binding globulin.

(*ESR2*) is broadly expressed. The two *ESR* have distinct functions and act antagonistically in multiple pathways: *ESR1* play an important role in growth and proliferation, while *ESR2* exerts anti-proliferative, differentiative and apoptotic actions in breast, prostate and colon cells [8]. Both receptors are required for the maintenance of glucose homeostasis with *ESR2* predominating in the skeletal muscle and *ESR1* in the adipose tissue [8,9].

DNA methylation is nowadays the best-defined epigenetic modification and involves inherent and acquired gene transcription changes, which occur independently of the DNA sequence [10,11]. *AR*, *ESR1* and *ESR2* promoter methylation are involved in the development of many diseases, such as atherosclerosis and tumorigenesis [12]. *AR* promoter methylation is implicated in the pathogenesis of prostatic cancer [13] and lung cancer [14], while *ESR1* is involved in breast cancer [15], hepatocellular carcinoma [16], colorectal carcinoma [17] and stomach cancer, and *ESR2* in endometriosis [8,18]. However, little is known about changes in the context of DNA methylation in these promoters upon CHT.

Changes in the *AR* expression associated to CHT may lead to changes in the sex hormones action in relation to the original sex and the desired sex. Sader's study described in a short group of FtoM GD individuals the effects on AR expression [19].

Given these data, we postulate that CHT for sex reassignment in GD could drive changes at the *AR*, *ESR1* and *ESR2*, affecting the DNA methylation and mRNA expression pattern of these receptors, that may influence in the phenotypical changes associated to CHT.

2. Material and methods

2.1. Study design and subjects

We carried out a prospective observational study on individuals with a diagnosis of GD, who were attended in the Identity Gender Unit (UIG) of the Hospital Clinic from July 2012 to November 2013. All