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ORIGINAL ARTICLE

Safety of gender affirming treatment in assigned female at birth transgender people and association of androgen and estrogen β receptor polymorphisms with clinical outcomes

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Abstract

Purpose Gender affirming hormone treatment (GAHT) with androgens in assigned female at birth (AFAB) people with Gender Incongruence (GI) can induce and maintain variable phenotypical changes, but individual response may be genetically determined. To clarify the role of AR and ERβ polymorphisms we prospectively evaluated AFAB subjects undergoing virilizing GAHT.

Methods Fifty-two AFAB people with confirmed GI were evaluated before (T0) and after 6 (T6) and 12 months (T12) of testosterone enanthate 250 mg i.m. every 28 days. Hormone profile (testosterone, estradiol), biochemical (blood count, glyco-metabolic profile) and clinical parameters (Ferriman-Gallwey score, pelvic organs) were evaluated at each time-point, as well as number of CAG and CA repeats for AR and ERβ, respectively.

Results All subjects have successfully achieved testosterone levels within normal male ranges and improved their degree of virilization, in absence of significant side effects. Hemoglobin, hematocrit and red blood cells were significantly increased after treatment, but within normal ranges. Ultrasound monitoring of pelvic organs showed their significant reduction already after 6 months of GATH, in absence of remarkable abnormalities. Furthermore, a lower number of CAG repeats was associated with a higher Ferriman-Gallwey score post treatment and a higher number of CA repeats was associated with uterine volume reduction.

Conclusion We confirmed safety and efficacy of testosterone treatment on all measured parameters. This preliminary data hints a future role of genetic polymorphisms to tailor GAHT in GI people, but evaluation on a larger cohort is necessary as the reduced sample size could limit data generalization at this stage.

Keywords Gender incongruence · AFAB · CA repeats · CAG repeats · Ferriman-Gallwey score · Pelvic ultrasound

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Introduction

The ICD-11 refers to gender incongruence (GI) as a marked and persistent incongruence between an individual's experienced gender and the sex assigned at birth, encompassing a broad spectrum of transgender people (binary and non-binary) within its definition [1]. The distress resulting from the mismatching physical appearance and gender identity, further negatively affected by personal experiences (stigma and social discrimination), often leads to the need of psychological, medical and surgical intervention. The standards of care for GI involve the use of hormone treatments (Gender Affirming Hormonal Therapy - GAHT) to develop and maintain the desired sexual characteristics. GAHT for transgender assigned female at birth (AFAB) people is based on one of the testosterone formulations available, including parenteral esters injections (testosterone enanthate or undecanoate) or transdermal formulations [2]. This virilizing treatment is aimed at interrupting the period, increasing the production of body and facial hair, increasing lean body mass. Further androgen related effects are the lowering the tone of the voice, the redistribution of body fat, the hypertrophy of the clitoris, and the increased libido [2]. The desired effects can range from an androgynous presentation to a full masculinization, but the subjects should be informed about the chronicity of the therapy, the potential loss of fertility, the side effects and the individual variability of treatment response [1]. In fact, androgens can induce variable phenotypical changes depending on androgen and estrogenic signaling, which are, on turn, dependent from the type of molecule and dosage used but also from an individual response that may be genetically and epigenetically determined [3]. Genetic polymorphisms can influence receptor sensitivity and signal transduction. In particular, androgen receptor (AR) presents short CAG (cytosine-adenine-guanine) repetitions in tandem and estrogen receptor-β (ERβ) presents short CA (cytosine-adenine) repetitions in tandem. The AR gene contains within the Exon 1 a stretch of CAG repetitions whose number influences the function of the receptor: a reduced number of repetitions is associated with an increase in signal transduction and consequently to the exogenous effect of testosterone [4]. Similarly, variants in ERα (TA repetitions) and ERβ (CA polymorph repetitions) are associated with different pathologies and bone mineral density, as well as variations in testosterone concentration [5, 6]. CA repeats in particular are located in intron 5 of the ERβ gene and it is expressed in female reproductive organs [7]. Even if its location is untranslated, it can apparently affect gene expression as women with fewer CA repeats have higher testosterone (total and free) and lower SHBG levels compared to women with a higher number of CA repeats, suggesting that the lower CA repeats variant leads to a less active receptor [5, 6]. Epigenetic regulation in response to testosterone administration might also play a relevant role in the modulation of phenotypical changes after testosterone treatment [3]. Since there is paucity of data on the role of AR and ERβ polymorphisms on biochemical and phenotypical changes in AFAB people undergoing virilizing GAHT, we conducted a prospective study of a group of AFAB people with GI before (T0) and after 6 (T6) and 12 months (T12) of testosterone administration. Our main outcomes were:

- the evaluation of the hormone profile (testosterone and estradiol levels), biochemical (blood count and glicometabolic profile) and clinical parameters (anthropometric measures, body composition, pelvic organs) in terms of both effectiveness and safety.
- the investigation of the possible modulation of the abovementioned clinical parameters by AR and ERβ functional polymorphisms.

Material and Methods

Written informed consent was obtained from all study participants and the study was conducted in accordance with the Declaration of Helsinki; the study protocol was approved by Ethical Committee "Sapienza" (Prot. 1057/2021). We selected trans AFAB people referring to the Gender Incongruence Outpatient Clinic of the Department of Experimental Medicine (Policlinico Umberto I - "Sapienza" University of Rome) for GAHT. Presence of GI was confirmed by mental health specialists (Servizio di Adeguamento tra Identità Fisica e Identità Psichica – SAIFIP, AO San Camillo Forlanini), according to DSM-V criteria. Inclusion criteria for recruited subjects were age 18 years or older and confirmed GI. Subjects with previous genital surgery and/or previous hormone treatment, as well as those uncompliant to follow up visits, were excluded. All subjects underwent medical history and physical examination including Ferriman-Gallway score (FGS) and pelvic ultrasound evaluation; furthermore, each subject provided a blood sample for routine biochemistry, hormone analyses and AR/ERβ gene sequencing. Additional hematological and biochemical data were retrieved from subject's medical records. All analyses were performed at baseline prior of testosterone administration (T0) and after 6 (T6) and 12 months (T12) of testosterone administration (intramuscular Testosterone enanthate 250 mg every 28 days).

Hormone profile analysis

A peripheral blood sample was collected from each subject between 8.00 and 9.00 a.m. after overnight fasting to measure serum levels of total testosterone and estradiol. Blood was centrifuged after 30 min and the serum was immediately frozen at −20 °C. Serum testosterone and estradiol

were quantified by Chemiluminescent Microparticle ImmunoAssay (CMIA, Architect System; Abbott Laboratories, Abbott Park, IL, USA), with detection limits of 0.28 nmol/L and 10 pg/mL, respectively. Intra- and inter-assay coefficients of variation were 2.1 and 3.6% at 10.08 nmol/L (testosterone) and 5 and 7% at 190 and 600 pg/mL (E2). Normal ranges for adults in our laboratory were 9.4–33.5 nmol/L (testosterone) and 24–108 pg/mL (E2).

DNA Extraction and AR and ERβ polymorphisms

DNA was extracted from peripheral blood leukocytes using Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Extracted DNA was quantified by NanoDrop ND-2000 (Thermo Fisher Scientific, Waltham, MA, USA). For Androgen Receptor, located on X chromosome, analysis of the percentage of inactivation of the alleles was performed as proposed by Zitzmann et al. (2004) [8]. To determine the length of the polymorphisms, CAG and CA repeats were analyzed by fragment analysis (3500 Genetic Analyzer, Applied Biosystems). These methods were previously described [3].

Pelvic ultrasound evaluation

Pelvic US evaluation was performed in the Prenatal Diagnosis Centre of the Department of Obstetrics, Gynaecology and Urologic Science of Sapienza, University of Rome. Baseline US evaluation was performed in early follicular phase (3rd - 5th day of the menstrual cycle), according to ASRM-AIUM [9, 10]. US scans were all conducted after proper preparation with a trans-abdominal convex probe to avoid the exacerbation of genital dysphoria (reported by all subjects). All examinations were conducted by the same operator and with the same device, thus limiting measurement bias. Uterus, endometrium and ovaries were evaluated in accordance with current literature and guidelines [10, 11]. The uterine and ovarian volume were obtained using the volume formula of an ellipsoid: Volume (cm3) = length (DL) x depth (DAP) x width (DT) x 0,523 or using VOCAL reconstruction, an 3d scan was performed and the volume was calculated on a 3d virtual rotation. The volumes calculated per uterus and ovaries were compared longitudinally by examining the variations between T0, T6 and T12, with the reference graphs by age and with the values obtained during therapy at T6 and T12.

Statistical analysis

The statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) 27.0 (SPSS Inc., Chicago, USA) software. Continuous variables were expressed as a mean ± standard deviations or medians and interquartile range, where appropriate, in relation to the normality of the value distributions evaluated with the Kolmogorov–Smirnov test. Comparisons among the baseline (T0), T6 and T12 values were computed through repeated measures ANOVA or Friedman's test, where appropriate. The results from multiple comparisons were Bonferroni adjusted post hoc. Categorical variables, expressed as frequencies or percentages, were confronted using the Fisher's exact test. Correlations were computed using Pearson's or Spearman's correlation test. A two-tailed p-value lower than 0.05 was considered as statistically significant. Categorical variables, expressed as percentages, are evaluated with the χ^2 test. To evaluate the effect of polymorphisms of AR and ER receptors on pelvic organs, univariate linear models have been constructed using organ measurements (uterus, ovary, endometrium) as a dependent variable and CAG/CA repeats as independent variables.

Results

We recruited 52 AFAB people with confirmed GI aged 24.8 ± 8.6 years (range 18–49 years) who started virilizing GAHT with testosterone enanthate 250 mg, injected i.m. every 28 days. It is worth stressing that the therapy was chosen according to the will of the subjects enrolled: all enrolled AFAB people did not report a non-binary identity and all were looking for a complete masculinization. The mean number of CAG repeats in our caseload was 22.4 ± 2.0 (range: 12–28), while the mean number of CA repeats was 22.3 ± 1.5 (range: 17–26). In particular, all subjects were heterozygous for AR polymorphism and the percentage of X inactivation was random in the majority of subjects (random inactivation: 49/52; skewed inactivation: 3/52). Regarding ERβ polymorphism, 53.8% (28/52) of the population was homozygous. Supplementary Fig. 1 shows the distributions of the mean CAG and CA repeats in our caseload.

Effectiveness and safety outcomes

Phenotype changes

During the follow up, subjects have improved their degree of virilization, measured as an increase in FG total scores detectable already at T6 (Fig. 1) (Bonferroni-Adjusted p values T0 vs. T6 and T0 vs. T12, both $p < 0.001$). When considering FG sub-scores, we recorded a homogeneous increase of hairs after the start of the treatment at T6 which remained stable at T12. While a weak trend of score increase was observable for thighs, upper arms and lower abdomen, no statistically significant increase in any specific

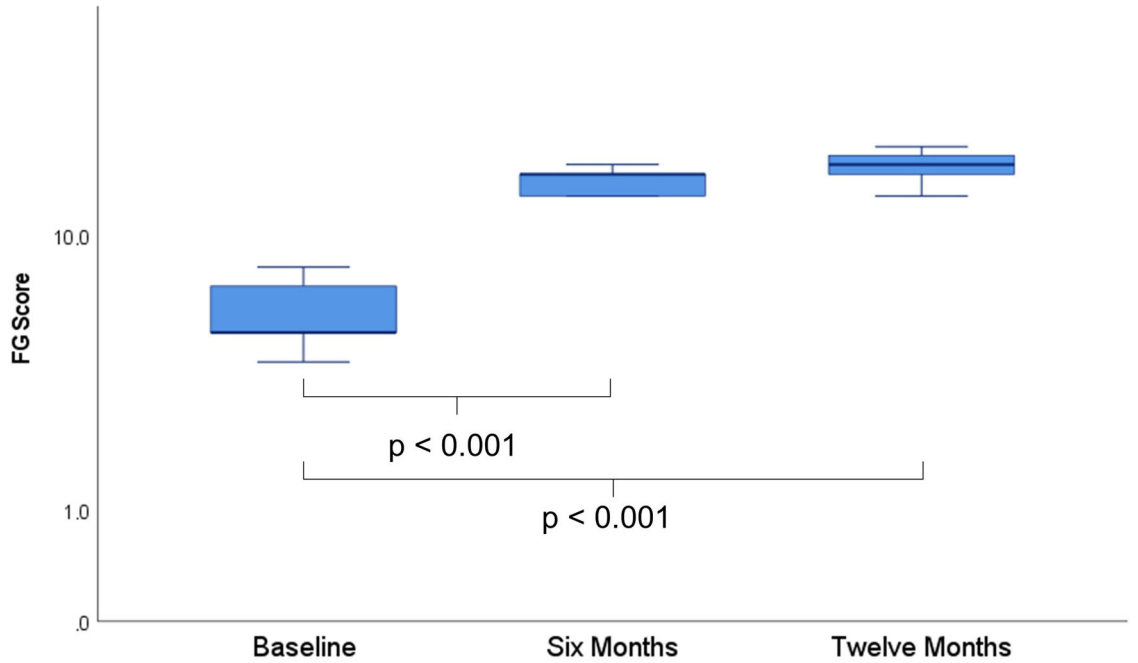

Fig. 1 Boxplots of Ferriman-Gallwey scores at baseline (T0) and Six (T6) and Twelve (T12) months after start of androgen GAHT treatment. Friedman's test. *P*-values are adjusted for multiple comparisons (Bonferroni)

body area compared to the others was detected. After adjusting for BMI and age, total testosterone level was the main parameter associated with phenotypical changes (OR 1.23; 95% CI 1.14–1.33, $p < 0.001$).

Hormone profile, biochemistry and blood cells count

Baseline total testosterone and estradiol levels were within biological female age-adjusted normal ranges. Administration of intramuscular testosterone increased total testosterone concentration at T6, while estradiol values at the same time point were reduced; these values remained constant after 12 months of treatment. We could not detect a significant change in FSH levels, but LH significantly reduced at T12 (Table 1). LH levels were correlated with FG scores (Spearman's $\rho = -0.209$; $p = 0.009$) and post treatment uterine volume (Spearman's $\rho = 0.187$; $p = 0.019$). Table 2 shows that hemoglobin (Hb) levels and hematocrit (Hct) were significantly higher at T6 and T12 compared to baseline. Glycolipid profile (blood glucose, HbA1c, total cholesterol, HDL, LDL and triglycerides), liver and kidney function at T0 were within the normal reference range provided by our laboratory. We could detect significantly increased concentrations of HDL and γGT at T6 only and increased levels of serum creatinine at both T6 and T12, but all these values remained well within normal ranges (Supplementary table 1). Univariate analyses confirmed a primary role of the increased testosterone levels in the detected changes in the blood cell count and glycolipid profile ($p < 0.001$).

Table 1 Total testosterone, estradiol, FSH and LH levels in AFAB subjects before (T0) and after (T6, T12) testosterone treatment

	Total Testosterone (nmol/L)	Estradiol (pg/mL)	FSH (mIU/ml)	LH (mIU/ml)
T0	1.38 ± 0.97 *1.20* (0.82–1.50)	96.7 ± 91.3 *64.05* (35.02–108.95)	6.6 ± 7.1 5.4 (4.0–6.6)	8.4 ± 4.9 5.0 (6.9–11.0)
T6	12.69 ± 5.5[a] *12.30* (9.82–14.40)	52.0 ± 33.4[a] *42.20* (33.47–57.40)	5.8 ± 2.6 5.2 (4.4–6.5)	7.5 ± 4.0 5.7 (4.6–8.7)
T12	12.36 ± 4.9[a] *12.10* (11.10–12.85)	52.0 ± 33.4[a] *39.50* (34.00–60.00)	5.8 ± 6.0 4.8 (3.7–5.9)	6.6 ± 4.7[b] 4.8 (3.8–7.6)
***p*-value**	<0.001	<0.001	0.628	0.008

[a]$p < 0.001$ *vs.* T0 (Bonferroni-adjusted)

[b]$p < 0.010$ *vs.* T0 (Bonferroni-adjusted)

Data are presented as means ± standard deviations, median (in italics), interquartile range (in brackets). Friedman's test

Pelvic ultrasound

Baseline ultrasound parameters were in the biological range for cisgender women. However, a significant decrease of uterus and ovarian volume, as well as of endometrial thickness was detected at T6 and persisted stable at T12 (Table 3).

Associations of outcomes with investigated polymorphisms

Finally, we were able to detect several significant associations of AR and ERβ polymorphisms with potential impact on clinical outcomes:

Table 2 Hemocromocytometric parameters in AFAB subjects before (T0) and after (T6, T12) testosterone treatment

	Red Blood Cells (x10^6/ml)	Hemoglobin (g/dl)	Hematocrit (%)	Platelet (x 10^3/ml)	MPV (fl)	White Blood Cells (x10^3/ml)
T0	4.6 ± 0.5 *4.5* (4.2–4.8)	13.3 ± 1.2 *13.4* (12.4–14.1)	40.1 ± 3.4 *39.8* (38.0–42.3)	263.9 ± 71.0 *260.0* (214.0–300.5)	8.8 ± 0.9 *8.6* (8.1–9.4)	7.1 ± 2.1 *6.5* (5.6–8.4)
T6	5.0 ± 0.5[a] *4.9* (4.5–5.3)	14.2 ± 1.1[a] *14.3* (13.7–14.7)	43.4 ± 2.8[a] *43.1* (41.9–45.0)	253.1 ± 49.9 *255.0* (218.0–284.0)	8.8 ± 1.0 *8.6* (8.3–9.5)	7.1 ± 2.8 *6.8* (5.7–8.0)
T12	5.0 ± 0.5[a] *5.1* (4.7–5.3)	14.4 ± 0.9[a] *14.5* (13.9–15.1)	44.1 ± 2.7[a] *44.7* (42.3–46.1)	255.9 ± 41.9 *254.0* (220.0–285.5)	10.5 ± 1.6 *8.9* (8.3–9.6)	6.6 ± 1.5 *6.5* (5.5–7.5)
P-value	<0.001	<0.001	<0.001	0.646	0.882	0.516

[a]$p < 0.001$ vs. T0 (Bonferroni-adjusted)

Data are presented as means ± standard deviations, median (in italics), interquartile range (in brackets). Friedman's test

Table 3 Comparisons of US measurements of pelvic organs

	Uterus (ml)	Endometrium (mm)	Right ovary (ml)	Left ovary (ml)
T0	50.9 ± 19.2 *46.8* (36.7–58.7)	5.1 ± 3.2 *4.9* (2.7–6.5)	6.5 ± 4.7 *5.0* (3.0–8.6)	6.9 ± 5.6 *5.7* (4.0–8.6)
T6	41.9 ± 18.5[a] *37.5* (28.8–50.2)	2.7 ± 2.1[c] *2.4* (1.0–4.0)	3.8 ± 4.5[b] *2.5* (1.7–4.3)	3.2 ± 1.8[b] *2.8* (2.1–4.3)
T12	38.8 ± 11.7[b] *38.6* (29.1–45.7)	1.9 ± 1.4[b] *2.0* (0.5–3.0)	2.7 ± 1.7[b] *2.3* (1.5–3.3)	2.6 ± 1.7[b] *2.1* (1.5–3.3)
P-value	<0.001	<0.001	<0.001	<0.001

[a]$p = 0.001$ vs. T0 (Bonferroni adjusted)

[b]$p < 0.001$ vs. T0 (Bonferroni adjusted)

[c]$p < 0.05$ vs. T0 (Bonferroni adjusted)

Data are presented as means ± standard deviations, median (in italics), interquartile range (in brackets). Friedman's test

Phenotype changes

Notably, a shorter polymorphic CAG repeats tract (≤22), corresponding to a more active receptor transcriptional activity, was significantly associated with development of FG score above the median of the population (median FG score: 14) after treatment (OR 3.07; 95% CI 1.32–7.1, $p = 0.009$).

Hormone profile, biochemistry and blood cells count

Moreover, regarding hormone levels, linear models showed a positive association between testosterone levels and number of CA repeats (ERβ) ($b = 0.82$, 95%CI 0.15–1.49; $p = 0.017$); however, and a positive trend between total testosterone and CAG repeats did not reach statistical significance (AR) ($b = 0.46$, 95%CI −0.04–0.96; $p = 0.070$). On the other hand, even after adjustment for the time from the start of the treatment, no significant association was found between glyco-metabolic parameters and the length of AR and ERβ polymorphisms (CAG repeats and CA repeats, respectively); likewise, the positive trend observed between CAG repeats and Hemoglobin concentration did not reach statistical significance ($p = 0.076$).

Pelvic ultrasound

Results from the univariate models showed that, despite the main variable significantly associated with uterine, endometrium and ovarian reduction was serum testosterone, we could determine that a higher number of CA repeats was clearly associated with uterine volume reduction (Table 4a–c). No significant association was detected between androgen receptor gene polymorphism with ultrasonographic variations in pelvic organs.

Discussion

Among known androgen and estrogen receptors polymorphisms, CAG repeats for the AR and CA repeats for ERβ have received particular attention due to associations with several diseases and conditions [5, 12, 13]. The rationale for their involvement in pathologies lies in their possible role in modulating receptor activity through modifications of the gene transcriptional activity [13–15]. Androgen receptor CAG polymorphic trait has been extensively studied in various pathological conditions and in men a low number of repetitions have been associated with increased risk of prostate hyperplasia and cancer, rheumatoid arthritis and infertility [5]. In cis-gender women there is fewer, but associations with acne, androgenetic alopecia, bone mass density, and breast cancer are known [5]. Regarding ERβ polymorphism, literature data reports associations in biological women between higher number of

Table 4 a - Coefficients from univariate linear model predicting uterine volume (ml) and significance of unadjusted and age-adjusted model. b - Coefficients from univariate linear model predicting endometrium thickness (mm) and significance of unadjusted and age-adjusted model. c - Coefficients from univariate linear model predicting mean ovarian volume (ml) and significance of unadjusted and age-adjusted model

	Coefficient	95% CI	*p*-value	*adj. p*-value
a				
Total testosterone	−0.57	−0.97 to −0.16	**0.006**	**0.002**
Estradiol	0.03	−0.01 to 0.07	0.190	0.435
CA repeats	10.56	3.20–17.91	**0.005**	**0.020**
CAG repeats	5.74	−1.71 to 13.19	0.131	0.149
b				
Total testosterone	−0.11	−0.17 to −0.05	**<0.001**	**<0.001**
Estradiol	0.12	0.01–0.18	**<0.001**	**<0.001**
CA repeats	0.979	−0.10 to 2.06	0.075	**0.048**
CAG repeats	0.590	−0.51 to 1.69	0.290	0.267
c				
Total testosterone	−0.19	−0.27 to −0.11	**<0.001**	**<0.001**
Estradiol	0.01	−0.01 to 0.01	0.224	0.144
CA repeats	0.835	−0.66 to 2.33	0.273	0.180
CAG repeats	0.795	−0.72 to 2.31	0.303	0.275

Bold values $p < 0.001$

CA repeats and bone mineral density [16] and higher testosterone levels [5]. In general, a longer polymorphic trait may correspond to a higher receptor function [6]. As expected, baseline estradiol and total testosterone concentrations in our recruited AFAB people were in the biological female range and at T6 total testosterone concentrations reached the eugonadic male range and persisted at T12, in accordance with good clinical practice [1, 2]. Likewise, estradiol significantly decreased at T6 and persisted constant at T12. Probably, aromatization of testosterone enanthate prevented a massive estradiol reduction although these levels do not seem to thwart the virilization process. As a matter of fact, the persistence of constant estradiol levels may even exert a protective effect on the glyco-metabolic profile [17, 18]. Regarding gonadotropins, FSH levels were not significantly changed by treatment, while LH showed a limited (but significant) reduction at T12. These relatively minor changes, while apparently in contrast with the phenotype changes, should probably be monitored in the AFAB subjects undergoing GAHT. Although we observed different gonadotropin patterns than other studies with non-suppression of the gonadal axis and, in general, a lower reduction of LH levels [19, 20]. These studies, in accordance with our data, suggest that LH in particular, together with testosterone levels, might be used as a marker of androgenization in these people, as it correlates with both Ferriman-Gallway scores and post treatment uterine volume. The non-suppression of the gonadal axis could be explained by the fact that we deliberately targeted the low-normal testosterone normal range of cisgender males as our goal: this allowed a sufficient and observable degree of virilization with the lowering of LH levels, without their full suppression. A previous report from Castellano et al. (2015) [20] showed a quality of life (general and sexual) and body image perception comparable between AFAB persons with LH lower that 3 mUI/ml and those with LH between 3 and 10 mUI/ml after testosterone treatment, while LH higher than 23 mUI/ml were associated with a worse body image perception. It is likely that a testosterone treatment that keeps LH well within normal cisgender male range could be and adequate dosing, particularly when considering that, in our caseload, it did not correspond with treatment side effects, especially at the level of uterus and endometrium, but this should probably be monitored in a longer follow up.

A first relevant aspect of our study is the identification of a role of functional variants of hormone receptors as modulators of clinical and phenotypical changes, as we hypothesized in a previous pilot study [3]. The detection of a positive association between testosterone levels and CA repeats (ERβ), and as a positive trend with CAG repeats (AR) suggest that the action of testosterone is very likely modulated by the complex interaction of the hormone receptor network. There is a severe paucity of data in literature regarding this aspect, but if confirmed it might provide the biological basis for tailoring of the GAHT treatment. Alongside the action of testosterone, which is constantly and obviously confirmed as the *primum movens* of all changes, genetic polymorphisms of the androgen receptor are capable of influencing gene transcriptional activity appear to significantly influence the intensity of induced sexual characteristics. In particular, a number of CAG repeats ≤22 is associated with a three-fold chance of achieve an FG score higher than the median of our population, in accordance to results from the few studies available [6, 21]. Testosterone esters are indeed effective in inducing hair growth and sebum production increasing during masculinizing GAHT [22]. A previous study from Wierckx et al. recorded an increase in the FG scores from median of 0.5 at baseline to 12.0 after 12 months of virilizing GAHT, and almost all of the studied transgender men achieved FG scores > 8 (diagnostic for "hirsutism" in cis-gender females) [23]. In our study, it is worth stressing that all subjects had not undergone hair removal treatments in the 6 months before the first testosterone administration and baseline FG score was non-diagnostic for hirsutism in a biological woman. At T6 and T12 FG scores increased

compared to baseline but remained rather constant. According to available evidence the role of AR receptor polymorphism in this change is uncertain. Several studies suggest that in cis-gender women with shorter CAG traits were associated with higher total testosterone levels and increased risk of hirsutism [24–26]. Conversely, Calvo et al. observed that in their study neither CAG polymorphism in AR nor their pattern of skewed inactivation in the X chromosome were associated with clinical hirsutism [27]. All these studies involved cis-gender AFAB people dealing with endogenous androgenic disorders. It is possible that exogenous testosterone administration in GAHT might induce a stronger androgen signaling and the modulating role of these gene functional variants might be revealed. This observation however will require future validation. Other changes in blood cell count and glycolipid profile we detected were within normal ranges and mostly expected during treatment and reported in other studies [2, 28]. Predictably, we were able to attribute these modifications to testosterone administration since it is a regulator of erythropoiesis. Cis-gender men with hypogonadism or undergoing androgen deprivation therapy might present normocytic anemia [29–32] and hypogonadal men show increased levels of hemoglobin and hematocrit following androgenic treatment [30, 33]. Indeed, the increase in hemoglobin and hematocrit levels, also present in our caseload, is rather important in transgender AFAB population and require careful monitoring, especially if cardiovascular risk factors are present [34]. According to our linear models, the role of AR and ER polymorphism was definitely milder or absent. Probably, the reason for this lack of association is due to the presence of other unaccounted factors. The increase in the value of hematocrit and hemoglobin may not conditioned only by androgens, but also by the state of hydration, the physical activity, the erythropoietin receptor activity, etc. Testosterone, although the mechanisms are not fully understood, is able to alter the lipid profile by increasing the levels of total cholesterol, LDL cholesterol, triglycerides and decrease in HDL levels [35, 36]. In our study, according to a previous observation by Fernandez et al. [28], we found no biologically relevant increases in glycolipid parameters during the 12 months of treatment. This aspect highlights the biological safety of GAHT in healthy AFAB subjects. Despite the first year of treatment is considered to carry the highest risk of acute cardiovascular [2, 37], in the absence of severe comorbidities the cardiovascular risk profile of subjects undergoing GAHT does not seem to increase during treatment in the short term. Hormone receptors polymorphisms associations with cardiovascular events (myocardial infarction/stroke) have been object of debate [38, 39]. Nonetheless, AR polymorphism has been inversely associated with cardiovascular risk factors such as total and LDL cholesterol in both healthy and polycystic ovarian syndrome cis-gender women [40–42], and positively with HDL levels in cis-gender men [4, 43, 44]. However, we were unable to detect associations between these parameters and CAG/CA polymorphic traits in our caseload. Regarding testosterone impact on the liver, the rather stable values of transaminase in our caseload confirm results from a recent study where the risk of increased transaminases is likely to be modest and not clinically meaningful in treated transgender persons AFAB people [45]. Another relevant aspect of our study is the monitoring of pelvic organs. In our knowledge this is first longitudinal ultrasound observation trans AFAB people during testosterone therapy. Testosterone is again the main factor associated to modifications. In fact, AR expression has been documented in the ovaries [46], in fallopian tubes [47] and endometrium [48]. Recent studies have also highlighted the local action of androgens in modulation of its function of the female reproductive system [49]. In vivo, Chadha et al. showed in a long-term androgen administration in transgender men induces up-regulation of AR in the uterine stroma and myometrium compared to untreated individuals, suggesting an androgen feedback mechanism on the endometrium [50]. An old report showed that, in transgender men treated with testosterone, the endometrium appeared to proliferate and in two cases cystic hyperplasia was detected [51]. Loverro et al., studied 12 trans AFAB people in hormone therapy undergoing hysterectomy: two persons reported increased endometrial thickness and persistent ovarian activity despite testosterone-induced amenorrhea in four of them [21]. In our study, thickening of the endometrium was not detected in any subject, whereas its significant reduction was found at both T6 and T12. Regarding ovaries, androgens can induce follicular growth, as demonstrated by the association of PCOS with hyperandrogenism [5]. On the other hand, AR blockade induces a reduction in the number of follicles and ovarian size in women with PCOS [52]. In our study, we recorded a statistically significant decrease in ovarian volume. This scenario is consensual to testosterone-induced amenorrhea appearing, in our caseload, after a median of 1.2 months. Moreover, no AFAB people showed ovarian activity (absence of follicles on ultrasound examination). Finally, regarding uterus volume variations, basal US scans indicated that the average volume of the uterus was within the biological women same age range (all the AFAB trans people enrolled were nulliparous). After 6 and 12 months of GAHT, uterine volumes were significantly lower than basal values. The most interesting aspect was the presence of an association with CA repeats and with the combined effect of CA and CAG repeats in the univariate models. This may be explained by a complex interaction of both receptors. The size of the uterus increases as CA repetitions increase, highlighting the trophic action of estrogen signaling on the

uterus itself. On the other hand, the effect of androgenic stimulation and the overall alteration of the hormone axis could have cumulatively contributed to the uterine volume regression. Nonetheless, this complex interaction between genetic functional variants of hormone receptor still needs to be fully elucidated.

A strength of our research was that the study group was sufficiently homogeneous because all subjects were Caucasian and did not have any genetic, endocrinological pathologies or other conditions that could affect the study outcomes. The age of the recruited subjects was rather heterogeneous, but according to linear models it appeared to have limited effects on measured outcomes. Our most important limitation was the relatively small sample size that could limit generalization of our results, especially in regards of gene polymorphisms. However, it should be remarked that gender incongruence is a relatively rare condition in the general population (0.6% of the American population; 0.2–0.6% in the European population).

Conclusions

In our knowledge, this study represents the first attempt to evaluate the association between CAG/CA polymorphism and clinical outcomes of GAHT.

First of all, this paper confirms safety and efficacy of testosterone treatment:

- all AFAB people have successfully achieved testosterone levels within normal male ranges and improved their degree of virilization, in absence of significant side effects.
- hemoglobin, hematocrit and red blood cells were significantly increased after treatment, but within normal ranges; likewise, glycolipid profile and liver and kidney function remained within normal ranges.
- ultrasound monitoring of pelvic organs showed their significant reduction already after 6 months of GATH, in absence of remarkable abnormalities.

Furthermore, our data highlight a potential role of functional variants of hormone receptors in modulating phenotypical changes. In particular, the number of CAG repeats was clearly associated with the achieved FG score and CA repeats appeared to be associated with uterine volume and endometrial thickness after treatment. While these associations will undoubtedly require further validations, our data indicates that genetic variability play a role in the response of GAHT. The knowledge of genetic factors modulating effectiveness of GAHT could be helpful to tailor both monitoring and therapies, with the possibility of using a pharmacogenetic screening to predict the expected clinical success on specific phenotypical outcomes of different hormonal therapies, in order to achieve the maximum degree of therapeutic personalization.

Data availability

The data presented in this study are available upon reasonable request from the corresponding author.

Author contributions F.P., F.L., and D.P. contributed to the study conception and design. Data collection and analyses were performed by D.P., A.G., A.C.C., A.A., E.D.P., S.D.C., F.F., A.M., F.R., and L.S. The first draft of the manuscript was written by F.P. and G.S. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. D.P., F.L., V.G., A.G., and M.M. critically reviewed the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethical approval The study was conducted in accordance with the Declaration of Helsinki and approved by Ethics Committee "Sapienza" (Prot. 1057/2021, date of approval 30/11/2021).

Informed consent Informed consent was obtained from all subjects involved in the study.

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