Endocrine (2019) 65:692–706
https://doi.org/10.1007/s12020-019-02008-6

ORIGINAL ARTICLE

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Bone and body composition response to testosterone therapy vary according to polymorphisms in the CYP19A1 gene

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Received: 19 May 2019 / Accepted: 6 July 2019 / Published online: 19 July 2019

Abstract

Purpose To evaluate the influence of single nucleotide polymorphisms (SNPs) of *CYP19A1* on the response and susceptibility to side effects from testosterone therapy. This is a prospective, single-arm study of men with low-morning serum testosterone (<10.68 nmol/l) administered testosterone cypionate 200 mg intramuscularly every 2 weeks for 18 months.

Methods We measured areal bone mineral density (aBMD) and body composition by dual energy X-ray absorptiometry, tibial volumetric BMD and geometry by peripheral quantitative computer tomography, bone turnover markers by enzyme-linked immunosorbent assay, testosterone, and estradiol by liquid-chromatography/mass-spectroscopy, genotyping by microarray, *CYP19A1* expression by quantitative polymerase chain reaction, hematocrit and prostate-specific antigen (PSA).

Results We enrolled 105 men (40–74-years-old). SNPs rs1062033 and rs700518 were associated with significant differences in outcomes at 18 months. The GG genotype in rs1062033 had significant increase in whole body aBMD, but had significant decrease in tibial bone size compared to the CG and CC genotypes. Body composition analysis showed that the CC genotype of rs1062033, and the AA genotype of rs700518, had significant increase in total lean and appendicular lean mass compared to CG and GG, and AG and GG, respectively. The GG genotype of rs700518 had significant increase in PSA (GG = 105.8 ± 23.3% vs. AG + AA = 53.4 ± 11.3%, $p = 0.046$) while hematocrit changes were comparable among genotypes. *CYP19A1* expression was highest in GG genotype in both SNPs.

Conclusions For the first time, we demonstrated that *CYP19A1* SNPs influence response to testosterone therapy in hypogonadal men, highlighting the importance of genetic profiling in therapeutics even for common clinical conditions.

Keywords CYP19A1 · Aromatase · Testosterone · Bone mineral density · Body composition

Introduction

Results from testosterone studies in hypogonadal men have suggested conflicting benefits and risks from the therapy [1–3]. Furthermore, studies suggested that certain factors, such as the degree of testosterone reduction, may influence the response to treatment [4]. However, none of the studies have evaluated the role of genetics on the response to testosterone therapy in men with hypogonadism. The class of CYP450 enzymes are responsible for gonadal hormone metabolism with activities reported to vary according to polymorphisms of the encoding genes [5, 6].

Like postmenopausal women, conversion of androgens to estrogen by aromatase enzyme represents the main source of estrogen production in men. Recently, estrogen has emerged as the main gonadal hormone regulating the male skeleton [7, 8]. This notion first came from observations that aromatase deficient males have increased height from

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Supplementary information The online version of this article (https://doi.org/10.1007/s12020-019-02008-6) contains supplementary material, which is available to authorized users.

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inability to close the epiphysis and suffer from osteoporosis. Both conditions were corrected by estrogen treatment [9]. Moreover, epidemiologic studies have demonstrated that in males, the correlation between estradiol with bone loss and fractures is stronger than that of testosterone [7, 10]. *CYP19A1* encodes for aromatase. Given the crucial role of estrogen in male skeletal health, reports of differences in bone mineral density (BMD), bone loss [11] and risk for fractures [12, 13] among the different *CYP19A1* variants in both genders are not surprising. Whereas response to estrogen replacement therapy was reportedly influenced by polymorphism in the *CYP19A1* [14], there are no reports suggesting that these polymorphisms also influence response to testosterone therapy in hypogonadal men. We hypothesize that polymorphisms in *CYP19A1* are associated with differences in musculoskeletal response and susceptibility to side effects from testosterone therapy due to variable enzyme activity resulting in different rates of testosterone to estradiol conversion. The primary objective of this study is to evaluate the influence of polymorphisms in *CYP19A1* on the musculoskeletal response and susceptibility to side effects from testosterone therapy.

Material and methods

Study design

This is a single arm, open label clinical trial in men with low-serum testosterone (<300 ng/dl or 10.68 nmol/L) given testosterone cypionate for 18 months (NCT01378299). This study was conducted at the New Mexico VA Health Care System (NMVAHCS) and the Michael E. DeBakey VA Medical Center (MEDVAMC) in accordance with the Declaration of Helsinki guidelines for the ethical treatment of human subjects. The protocol was approved by the University of New Mexico and Baylor College of Medicine Institutional Review Board. A copy of the protocol is available online (Supplement_Protocol_Final_Version). All patients provided written informed consent.

Study population

A total of 342 male veterans attending the Endocrine, Urology and Primary Care Clinics of the NMVAHCS and MEDVAMC were screened for the study. Inclusion criteria: males, 40–75-years-old, and with average morning fasting total serum testosterone of <10.68 nmol/L (<300 ng/dl) from samples taken twice at least 30 min apart between 0800 and 1100 hours [15–18]. Exclusion criteria: treatment with bone-acting drugs (e.g. bisphosphonates, glucocorticoids, sex-steroids, selective estrogen receptor modulators, androgen deprivation therapy, and anticonvulsants), and finasteride, osteoporosis, diseases affecting bone metabolism such as: hyperparathyroidism, chronic liver disease, uncontrolled/untreated hyperthyroidism, and significant renal impairment (creatinine of >132.63 µmol/L), history of prostate and breast cancer, untreated sleep apnea, and conditions that may prevent the subject from finishing the study. The study was monitored by the Veterans Affairs Clinical Science Research and Development Data Monitoring Committee, which convened three times a year.

Testosterone therapy

Testosterone cypionate was initiated at a dose of 200 mg every 2 weeks and adjusted to a target serum testosterone level between 17.3–27.7 nmol/L (500–800 ng/dl). However, after the 3rd year of the study, upon the direction of the FDA, this target was changed to 17.3–20.8 nmol/L (300–600 ng/dl). The data in the last 6 months of 16 subjects at NMVAHCS and all 15 subjects at MEDVAMC were affected by the change. Comparing testosterone levels at different timepoints showed no significant differences between those who were and those who were not affected by the change (Supplementary Table 1) except at 6 months where levels are higher for those affected. Fifty-one participants at NMVAHCS did self-injections, 38 received injections from the study team only, while two started with the study team and later did self-injections. Five subjects at MEDVAMC received injections from the study team and 10 did self-injections.

Dosage adjustments of testosterone cypionate injections were made according to results of serum testosterone and hematocrit levels from serial blood testing obtained as part of the efficacy and safety monitoring. A decrease in the dose was done for patients who develop a hematocrit of >52%. Repeat testosterone measurement was performed 2 months after a dose change, including a repeat hematocrit for those with elevated hematocrit. Otherwise, testosterone levels were measured at baseline, 3, 6, 12, and 18 months. Safety monitoring included assessment of prostate specific antigen (PSA), hematocrit, lipid profile, and liver enzymes at baseline, 3, 6, 12, and 18 months.

Body mass index

Body mass index (BMI) calculated as weight (kg) divided by the square of the height (m^2). Height and weight were measured using a standard stadiometer and weighing scale, respectively.

Areal BMD

Areal BMD (aBMD) of the lumbar spine, proximal left femur, and whole body (WBBMD) were measured at baseline, 6, 12, and 18 months by dual energy X-ray

absorptiometry (DXA) using Hologic Discovery (Hologic Inc, Bedford, MA, USA). Regions of interest in the femur include total hip and femoral neck. The coefficients of variation (CV) at our center are ~1.1% for the lumbar spine and ~1.2% for the proximal femur [19].

Volumetric BMD

Volumetric bone mineral density (vBMD) and bone geometry were assessed by peripheral quantitative computer tomography (pQCT) on the left tibia at baseline, 6 and 18 months using Stratec XCT-2000 (Stratec GmbH, Pforzheim, Germany) as previously described [20]. Trabecular bone parameters were obtained at the distal 4% of the tibia and cortical parameters at the 38% of the tibia. The CV for these measurements in our center is 0.96%. Since pQCT machine was not available at the start of the study, only 88 subjects were able to participate in this testing.

Body composition

Total fat mass (TMF), truncal fat (TrF), total lean mass (TLM) and appendicular lean mass (ALM) were measured by DXA at baseline, 6, 12, and 18 months as previously described [21].

Prostatic ultrasound

A prostatic ultrasound to assess changes in prostate volume was performed at baseline and 18 months. Urinary symptoms were assessed using the International Prostate Symptom Score questionnaire (IPSS) at baseline, 3, 6, 12, and 18 months.

Biochemical measurements

Screening serum testosterone was measured by immunoassay at the NMVAHCS and MEDVAMC clinical laboratories. At the end of the study, serum testosterone and estradiol were measured from samples collected at baseline, 3, 6, 12, and 18 months using liquid chromatography-mass spectroscopy (Mayo Clinic Laboratories, Rochester, MN). Hematocrit and PSA were measured at baseline, 3, 6, 12, and 18 months at the NMVAHCS and MEDVAMC clinical laboratories. The following were measured using enzyme-linked immunosorbent assay (ELISA) kits: C-terminal telopeptide of type I collagen (CTX, a marker of bone resorption) (Crosslaps; Immunodiagnostic System Inc., Gaithersburg, MD), osteocalcin, a marker of bone formation,(Metra OC; Quidel Corporation, San Diego, CA), and sclerostin (TECOmedical Sclerostin HS En-zyme Immunoassay Kit, Quidel Corp, San Diego, CA). at baseline, 6, 12, and 18 months.

Genotyping

Genomic DNA was extracted from peripheral leukocytes [22]. DNA samples were processed using the standard Affymetrix protocol (Affymetrix Genome-Wide Human SNP Nsp/Sty 6.0 User Guide, Rev 8.). SNPs for the CYP19A1 gene were selected based on: (1) their likelihood for success within the parameters of the assay, (2) their potential to be functionally active according to their location in the gene, (3) their previous associations with differences in estradiol levels, gene expression, transcriptional activity, (4) reported association with bone density and fractures. Genotypes for SNPs found to have significant differences in the outcomes evaluated were confirmed by allelic discrimination assay using specific TaqMan SNP Genotyping Assays as previously described [23].

CYP19A1 gene expression

CYP19A1 gene expression from buffy coat was performed by real-time quantitative polymerase chain reaction at baseline, 6, 12, and 18 months. RNA was extracted from buffy coat using RiboPure Blood (Invitrogen, #AM1928). Two-hundred nanograms of RNA were used for retrotranscription into cDNA and performed using SuperScript VILO Master Mix (Invitrogen, Carlsbad, CA, USA) following protocol instructions. FAM-labeled TaqMan Gene expression assays (Applied Biosystem, College Station, TX, USA) for *CYP19A1* (Assay ID: Hs00903411_m1) and VIC-labeled TaqMan gene expression assay for housekeeping *18S* (Assay ID: Hs03003631), and TaqMan Universal Master Mix were used following the manufacturer's protocol. Relative quantification: ΔΔCT relative quantification: gene expression of our sample vs gene expression of human control total RNA (Applied Biosystem #4307281) adjusted for housekeeping gene expression. Data analysis was performed using Real Time PCR system QuantStudio5 and QuantStudio Design & Analysis Software 1.3.1, respectively.

Statistical analysis

Based on the allelic frequency of rs2470152 in the *CYP19A1* gene [24], the sample size for genotypes GG ($n = 21$) and AG + AA ($n = 63$) was adequate to detect an 0.71 effect size in the critical comparison of spine BMD changes with 80% power and alpha = 0.05. Feasibility of this power analysis was argued based on the effect size reported in Amory et al. [25] and their inclusion of non-responders. Amory did not have genotyping but non-responders were expected to be mostly genotypes AG + AA; so the inclusion of genotyping was expected to result in the larger effect size (0.71) in our study. Because all

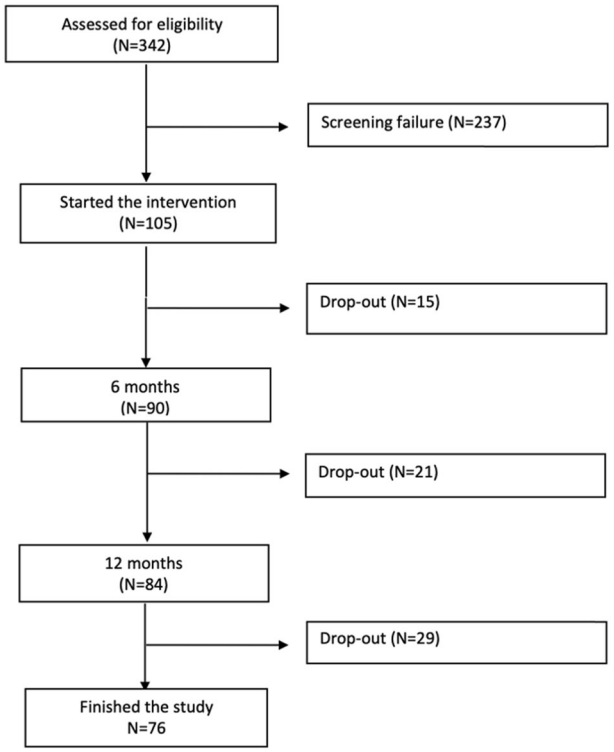

Fig. 1 Number of subjects screened, enrolled, and returned for follow-up at different timepoints. All the subjects received testosterone cypionate and responses were compared according to genotypes of the CYP19A1 gene polymorphisms

analyses were intention-to-treat, dropouts who had at least one follow-up data were included in the analysis.

Results are reported as means ± SD for text and tables and means ± SE for figures. P values < 0.05 were considered statistically significant. Baseline characteristics comparisons used analysis of variance or Fisher's exact test. Longitudinal changes between genotypes were tested using mixed-model repeated-measures analysis of variance (overall), and (post hoc) analysis of covariance (ANCOVA) of the percent change at each time (6, 12, 18 months) adjusting for baseline values, age, BMI. Analysis for changes in tibial pQCT parameters at 6 and 18 months were additionally adjusted for ALM at 6 months and 18 months, respectively. Analyses used SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA).

Results

One hundred and five men were enrolled, 90 returned for follow-up at 6 months, 84 at 12 months and 76 at 18 months (Fig. 1). The baseline general characteristics of the participants have been published elsewhere [21, 26]. Our analysis showed that two SNPs are associated with important clinical outcomes, the rs1062033 (C/G in exon 1.2) and rs700518 (A/G at Val80). One hundred and four subjects were successfully genotyped for rs1062033 and 103 for rs700518. Our analysis shows no significant differences in baseline characteristics (Table 1), aBMD, and body composition (Table 2) according to genotypes in both SNPs.

Bone outcomes

aBMD

There was a significant increase in spine aBMD in the entire population at 18 months compared to baseline (baseline = 1.124 ± 0.157 g/cm^2 vs. 18 months = 1.180 ± 0.179 g/cm^2, $p = 0.03$) but not in other skeletal sites. Compared to CC and GG genotypes for rs1062033, the increase in spine BMD is lesser in men with CG genotype at 12 months ($p = 0.006$) while the GG genotype had significant increases in WBBMD at 12 ($p = 0.03$) and 18 months ($p = 0.04$) compared to CG and CC genotypes (Table 3). For rs700518, those carrying the AG genotype experienced an increase in femoral neck BMD at 18 months compared to mild reductions among GG and AA genotypes ($p = 0.03$) (Table 3). Significant increase in WBBMD in the GG compared to the AA genotype at 6 months ($p = 0.04$) and to AA and AG genotypes at 12 months ($p = 0.05$) (Table 3) were also observed.

vBMD and bone geometry

There were no significant differences in vBMD and parameters of bone geometry and structure at all timepoints compared to baseline in the entire group (data not shown). There were also no significant intergenotype differences in the changes in these parameters in both SNPs with analysis adjusted for baseline values and age (Table 4, Model 1). However, individuals with GG genotype for rs1062033 experienced a significant decrease in bone size (total area), cortical area, and periosteal circumference at 6 and 18 months and in total mineral content, cortical content, and endosteal circumference at 18 months compared to no change or mild decrease in these parameters in CG and CC genotypes when ALM at 6 and 18 months, respectively, was included as additional covariate (Table 4, Model 2). Similarly, a trend for decrease in total area in GG compared to AG and AA genotypes of rs700518 was observed at 18 months after adjustment for ALM.

Bone turnover markers

There were no significant differences in CTX, osteocalcin, and sclerostin levels at all timepoints compared to baseline (data not shown). Genotype analysis showed continuous suppression of CTX (Table 5) in the CC genotype of rs1062033, with return close to baseline after a period of

Table 1 Baseline characteristics of the participants according to the rs1062033 and rs700518 polymorphisms of the *CYP19A1*

Rs1062033	CC ($N = 41$)	GC ($N = 45$)	GG ($N = 18$)	*P* value
Age (years)	58.6 ± 9.9	60.2 ± 7.3	59.8 ± 7.6	0.60
BMI (kg/m^2)	32.5 ± 4.6	32.8 ± 5.6	32.1 ± 7.1	0.91
Testosterone				
Immunoassay				
nmol/L	7.30 ± 2.4	7.41 ± 2.1	7.09 ± 2.0	0.87
ng/dL	212.2 ± 68.5	213.5 ± 61.5	204.1 ± 57.9	0.87
LC/MS				
nmol/L	9.42 ± 2.8	9.58 ± 3.0	9.16 ± 2.9	0.88
ng/dL	272.9 ± 80.3	276.0 ± 87.3	263.9 ± 83.6	0.88
Estradiol				
pmol/L	63.0 ± 28.5	61.3 ± 21.8	62.8 ± 9.1	0.94
pg/mL	17.2 ± 7.8	16.7 ± 5.9	17.1 ± 2.7	0.94
Rs700518	AA ($N = 21$)	GA ($N = 49$)	GG ($N = 33$)	*P* value
Age (years)	60.2 ± 9.7	59.5 ± 7.6	59.9 ± 7.5	0.94
BMI (kg/m^2)	32.6 ± 4.9	33.1 ± 5.4	31.6 ± 6.2	0.53
Testosterone				
Immunoassay				
nmol/L	7.12 ± 2.5	7.22 ± 2.3	7.66 ± 1.7	0.66
ng/dL	205.0 ± 72.8	207.9 ± 64.7	220.8 ± 48.6	0.66
LC/MS				
nmol/L	9.37 ± 3.1	9.16 ± 3.0	9.97 ± 2.6	0.58
ng/dL	269.8 ± 90.2	263.8 ± 86.0	287.1 ± 76.0	0.58
Estradiol				
pmol/L	60.6 ± 30.6	61.8 ± 22.0	65.0 ± 10.9	0.79
pg/mL	16.5 ± 8.3	16.8 ± 6.0	17.7 ± 3.0	0.79

LC/MS liquid chromatography/mass spectroscopy, *BMI* body mass index

suppression in the CG and increased in the GG genotype at 18 months ($p = 0.008$). CTX changes among the genotypes in the rs700518 followed the same direction as the rs1062033 genotypes, i.e. AA genotype suppressed all throughout the intervention period, returning close to baseline in AG and significantly increased at 18 months in GG genotype ($p = 0.05$) (Table 5). Meanwhile, there were no significant differences in the changes in osteocalcin and sclerostin levels in the genotypes of both SNPs.

Body composition

Compared to bassline, there was a significant reduction in TFM at 6 (−6.4 ± 6.7%, $p = 0.04$), 12 (−7.2 ± 9.1%, $p = 0.02$), and 18 months (−6.3 ± 9.2%, $p = 0.04$) and in TrF at 6 (−7.0 ± 8.8%, $p = 0.047$) and 12 months (−7.1 ± 10.8%, $p = 0.03$), with a trend for TrF loss at 18 months (−5.2 ± 11.6%, $p = 0.08$) in the entire group. Analysis according to rs1062033 genotypes (Fig. 2a, Supplementary Table 2) showed greater, but non-significant, loss of TMF in the CC compared to CG and GG genotypes at all timepoints. TrF loss was significantly greater in the CC compared to the CG and GG genotypes at 6 and to CG genotype at 12 months (Fig. 2b, Supplementary Table 2). TrF loss was maintained in both CC and CG, but was lost in the GG genotype at 18 months ($p = 0.14$). Meanwhile men with the rs700518 AA genotype experienced the greatest TMF (Fig. 2c, Supplementary Table 2) and TrF (Fig. 2d, Supplementary Table 2) loss at all timepoints compared to the AG and GG genotypes, but between-group differences were not significant.

A non-significant trend for higher TLM compared to baseline was seen in the entire group at 6, 12, and 18 months (data not shown). Meanwhile, ALM significantly increased from baseline at 6 (+5.6 ± 5.3%, $p = 0.04$) and 12 months (+5.0 ± 5.6%, $p = 0.03$) with a trend at 18 months (+3.9 ± 5.6%, $p = 0.09$) in the entire group. Analysis according to rs1062033, showed that men with the CC genotype experienced significantly greater increases in

Table 2 Baseline Areal bone mineral density and body composition according to genotypes of rs10620133 and rs700518

Rs1062033	CC	CG	GG	*P* value
Areal bone mineral density (g/cm^2)				
Spine	1.107 ± 0.16	1.118 ± 0.16	1.173 ± 0.16	0.32
Total hip	1.063 ± 0.12	1.091 ± 0.15	1.085 ± 0.15	0.66
Femoral neck	0.805 ± 0.12	0.840 ± 0.13	0.814 ± 0.13	0.42
Whole body	1.156 ± 0.10	1.167 ± 0.12	1.160 ± 0.14	0.91
Body composition (kg)				
Total body fat	32.93 ± 10.25	33.91 ± 11.21	30.65 ± 12.03	0.60
Truncal fat	17.58 ± 6.58	17.94 ± 6.83	16.28 ± 7.88	0.71
Total lean mass	64.39 ± 78.85	64.93 ± 7.22	62.67 ± 87.92	0.60
Appendicular lean mass	28.80 ± 4.44	28.99 ± 3.63	28.00 ± 4.78	0.71
Rs700518	AA	AG	GG	*P* value
Areal bone mineral density (g/cm^2)				
Spine	1.129 ± 0.17	1.128 ± 0.15	1.129 ± 0.16	0.99
Total hip	1.060 ± 0.15	1.092 ± 0.15	1.080 ± 0.11	0.68
Femoral neck	0.820 ± 0.11	0.832 ± 0.13	0.813 ± 0.13	0.83
Whole body	1.157 ± 0.14	1.156 ± 0.12	1.169 ± 0.14	0.89
Body composition (kg)				
Total body fat	33.04 ± 10.44	34.53 ± 11.65	30.27 ± 8.46	0.33
Truncal fat	17.65 ± 6.79	18.40 ± 7.18	15.93 ± 5.61	0.40
Total lean mass	64.32 ± 7.63	65.61 ± 7.09	62.26 ± 8.40	0.26
Appendicular lean mass	28.71 ± 4.18	29.39 ± 3.82	27.72 ± 4.41	0.31

Means ± SD

TLM compared to CG and GG genotypes at 18 months (Fig. 3a, Supplementary Table 2), and in ALM compared to CG and GG genotypes at 12 and 18 months, respectively (Fig. 3b, Supplementary Table 2). Of note, the initial increases in TLM and ALM at 6 and 12 months in the GG genotype, were lost at 18 months. TLM (Fig. 3c, Supplementary Table 2) and ALM (Fig. 3d, Supplementary Table 2) increased in all rs700518 genotypes, but significantly more in the AA genotype at all timepoints. Likewise, after an initial increase, both TLM and ALM were back to baseline at 18 months in the GG genotype.

Hormone levels

The increase in estradiol was significantly higher in the rs1062033 GG genotype at 18 months, but there was no difference in rs700518 genotypes (Table 6). There was a trend for greater increase in testosterone levels in the rs1062033 CC genotype and rs700518 AA genotype at 18 months (both $p = 0.06$).

Hematocrit, PSA, and prostate volume

Every genotype in rs1062033 and rs700518 had increases in PSA and hematocrit but no intergenotype differences in both parameters were observed (Table 7). However, dominant/recessive model analysis of PSA changes showed a significantly higher PSA increase in those without the A allele for rs700518 (GG = 105.8 ± 23.3%) compared to those with the A allele (AG + AA = 53.4 ± 11.3%) $p = 0.046$, at 18 months. There were no significant changes in prostate volume in the 44 subjects who underwent prostate ultrasound at baseline and 18 months and no intergenotype differences among the genotypes of both SNPs (Supplementary Table 3). There were no significant increase in IPSS scores in the entire population and no intergenotype differences in changes in IPSS scores.

Gene expression

Gene expression studies showed no significant changes in mRNA level between baseline and 18 months (baseline = 0.295 ± 0.60 vs. 18 months = 0.170 ± 0.32, p = NS) in the entire group. However, analysis by genotype shows significantly higher mRNA levels among those carrying the GG genotype for rs1062033 ($p = 0.0001$) and rs700518 ($p = 0.04$) at 18 months compared to their corresponding genotypes (Fig. 4a, b).

Linkage analysis

The genotype frequencies for both rs1062033 and rs700518 were in Hardy–Weinberg equilibrium in our population

Table 3 Changes (%) in the Spine, total hip, femoral neck, and whole body areal bone mineral density with testosterone therapy according to rs1062033 and rs700518 polymorphism of the *CYP19A1*

Rs1062033	CC	CG	GG	*P* value
Spine				
6 months	2.52 ± 0.62	1.59 ± 0.49	2.19 ± 0.82	0.42
12 months	3.38 ± 0.50[b]	1.58 ± 0.43	3.87 ± 0.76[a]	**0.006**
18 months	3.74 ± 0.63	2.85 ± 0.56	4.72 ± 0.91	0.19
Total hip				
6 months	0.70 ± 0.65	0.39 ± 0.56	−0.56 ± 0.98	0.52
12 months	0.42 ± 0.67	0.52 ± 0.58	−0.50 ± 1.06	0.69
18 months	0.64 ± 0.56	0.70 ± 0.50	0.33 ± 0.84	0.93
Femoral neck				
6 months	0.42 ± 0.87	−0.58 ± 0.75	0.39 ± 1.31	0.64
12 months	0.34 ± 0.74	−0.05 ± 0.67	−0.39 ± 1.19	0.74
18 months	0.22 ± 0.96	0.64 ± 0.81	−1.04 ± 1.31	0.52
Whole body				
6 months	0.31 ± 0.41	0.81 ± 0.34	1.75 ± 0.60	0.11
12 months	1.13 ± 0.39	0.47 ± 0.34	2.16 ± 0.60[a,c]	**0.03**
18 months	1.48 ± 0.46	1.38 ± 0.41	2.20 ± 0.67[a,c]	**0.04**
Rs700518	AA	AG	GG	*P* value
Spine				
6 months	3.13 ± 0.62	1.57 ± 0.43	1.71 ± 0.75	0.10
12 months	3.26 ± 0.56	2.05 ± 0.41	2.96 ± 0.74	0.22
18 months	3.82 ± 0.70	4.13 ± 0.52	3.69 ± 0.87	0.95
Total hip				
6 months	0.45 ± 0.98	0.51 ± 0.52	−0.26 ± 0.73	0.70
12 months	0.30 ± 0.74	0.50 ± 0.56	−0.15 ± 1.11	0.85
18 months	0.08 ± 0.66	0.93 ± 0.49	0.38 ± 0.93	0.70
Femoral neck				
6 months	−0.42 ± 1.37	−0.12 ± 0.72	0.21 ± 1.01	0.92
12 months	0.18 ± 1.18	0.03 ± 0.61	−0.11 ± 0.82	0.97
18 months	−1.25 ± 0.91	1.51 ± 0.69[d,e]	−1.09 ± 1.28	**0.03**
Whole body				
6 months	−0.02 ± 0.46	0.87 ± 0.30	1.63 ± 0.55[f]	**0.04**
12 months	1.02 ± 0.45	0.55 ± 0.31	2.08 ± 0.57[f,d]	**0.05**
18 months	1.16 ± 0.53	1.66 ± 0.38	2.22 ± 0.64	0.32

Data are expressed as Mean ± SD, Bold values are *p* values that are significant

P values adjusted for age and BMI; [a]$p < 0.05$ GG vs. CG, [b]$p < 0.05$ CC vs. CG, [c]$p < 0.05$ GG vs. CC, [d]$p < 0.05$ AG vs. GG, [e]$p < 0.05$ AG vs. AA, [f]$P < 0.05$ GG vs. AA

(https://wpcalc.com/en/equilibrium-hardy-weinberg/). Given the similarities in phenotypes between the two SNPs, r^2 was used to analyze for linkage between the two SNPs [27, 28], which revealed an r^2 of 0.22 suggesting that they are in weak linkage disequilibrium in this specific population.

Adverse events

There were seven cardiac events, two neurologic events, 10 hematologic events, and one urologic event among others. A detailed list of adverse events can be found in Supplementary Table 4. There were no deaths to report.

Table 4 Changes (%) in bone volumetric bone mineral density, bone geometry and bone structure by peripheral quantitative computer tomography with testosterone therapy according to the rs1062033 and rs700518 polymorphism of the *CYP19A1*

Rs1062033	CC	CG	GG	Model 1 *P* value	Model 2 *P* value
6 months					
Total density	−1.71 ± 1.14	1.66 ± 1.16	−0.30 ± 2.13	0.33	0.16
Total area	0.07 ± 0.97	−2.56 ± 0.99	−5.11 ± 1.81[a]	0.26	**0.04**
Total content	−1.69 ± 1.08	−1.11 ± 1.11	−5.56 ± 2.02	0.32	0.13
Cortical area	0.17 ± 0.58	−0.32 ± 0.59	−2.97 ± 1.08[a,b]	0.20	**0.04**
Cortical content	−1.49 ± 1.17	−0.46 ± 1.20	−5.09 ± 2.07	0.32	0.16
Cortical density	−1.74 ± 0.93	−0.14 ± 0.95	−2.18 ± 1.74	0.62	0.39
Cortical thickness	0.34 ± 0.79	1.88 ± 0.80	0.39 ± 1.47	0.34	0.37
Periosteal circumference	0.02 ± 0.49	−1.32 ± 0.50	−2.63 ± 0.91[a]	0.26	**0.04**
Endosteal circumference	−0.10 ± 1.26	−3.29 ± 1.28	−4.71 ± 2.34	0.33	0.15
18 months					
Total density	−2.03 ± 0.97	−1.01 ± 0.96	−1.67 ± 1.78	0.79	0.77
Total area	0.65 ± 0.99	1.66 ± 0.98	−5.90 ± 21.72[a,b]	0.06	**0.03**
Total content	−1.42 ± 0.99	0.43 ± 0.98	−6.72 ± 1.72[a,b]	0.13	**0.009**
Cortical area	−0.11 ± 0.56	−0.14 ± 0.55	−3.69 ± 0.97[a,b]	0.07	**0.007**
Cortical content	−1.59 ± 1.09	−0.31 ± 1.13	−6.50 ± 1.92[a,b]	0.21	**0.03**
Cortical density	−1.56 ± 0.83	−0.16 ± 0.81	−2.88 ± 1.43	0.60	0.28
Cortical thickness	−0.58 ± 0.80	−1.30 ± 0.79	−0.01 ± 1.39	0.94	0.69
Periosteal circumference	0.30 ± 0.58	0.79 ± 0.59	−3.01 ± 1.01[a,b]	0.06	**0.002**
Endosteal circumference	1.12 ± 1.34	2.62 ± 1.35	−5.19 ± 2.33[a,b]	0.16	**0.03**
Rs700518	AA	AG	GG	Model 1 *P* value	Model 2 *P* value
6 months					
Total density	−1.93 ± 1.36	1.44 ± 1.01	−1.17 ± 2.01	0.13	0.13
Total area	0.23 ± 1.26	−2.80 ± 0.94	−2.61 ± 1.87	0.36	0.15
Total content	−1.79 ± 1.33	−1.55 ± 0.99	−3.84 ± 1.97	0.92	0.56
Cortical area	−0.08 ± 0.80	−0.61 ± 0.59	−0.93 ± 1.18	0.91	0.78
Cortical content	−1.62 ± 1.46	−1.01 ± 1.09	−3.23 ± 2.16	0.83	0.63
Cortical density	−1.63 ± 1.10	−0.40 ± 0.82	−2.40 ± 1.63	0.80	0.45
Cortical thickness	−0.12 ± 0.99	1.70 ± 0.74	1.03 ± 1.47	0.17	0.33
Periosteal circumference	0.09 ± 0.64	−1.45 ± 0.48	−1.34 ± 0.94	0.35	0.15
Endosteal circumference	0.45 ± 1.62	−3.49 ± 1.21	−2.85 ± 2.40	0.20	0.15
18 months					
Total density	−2.16 ± 1.13	−1.14 ± 0.86	−1.62 ± 1.67	0.88	0.78
Total area	0.22 ± 1.21	1.21 ± 0.91	−3.86 ± 1.78	0.53	0.07
Total content	−0.73 ± 1.59	0.49 ± 1.13	−2.72 ± 2.18	0.53	0.49
Cortical area	−0.44 ± 0.68	−0.44 ± 0.51	−1.62 ± 1.01	0.84	0.61
Cortical content	−2.06 ± 1.24	−0.73 ± 0.93	−4.73 ± 1.83	0.68	0.23
Cortical density	−1.71 ± 0.97	−0.28 ± 0.73	−3.21 ± 1.43	0.73	0.23
Cortical thickness	−1.36 ± 0.94	−0.67 ± 0.70	1.10 ± 1.38	0.75	0.35
Periosteal circumference	0.08 ± 0.60	0.56 ± 0.45	−1.94 ± 0.89	0.54	0.08
Endosteal circumference	0.89 ± 1.60	2.17 ± 1.22	−4.15 ± 2.35	0.44	0.11

Data are expressed as mean ± SD; Bold values are *p* values that are significant. Model 1: adjusted for baseline age, baseline BMI, Model 2: Model 1 plus appendicular lean mass [a]$p < 0.05$ GG vs. CC, [b]$p < 0.05$ GG vs. CG

BMI body mass index

Table 5 Changes (%) in markers of bone turnover with testosterone therapy according to the rs1062033 and rs70518 polymorphisms of the CYP19A1

Rs1062033	CC	CG	GG	*P* value
CTX				
6 months	−33.86 ± 34.02	−17.45 ± 35.53	−9.0 ± 92.18	0.29
12 months	−16.00 ± 50.49	0.79 ± 42.25	−3.85 ± 53.62	0.40
18 months	−14.49 ± 51.05	5.89 ± 48.82	48.96 ± 98.42[a,b]	0.008
Osteocalcin				
6 months	−2.61 ± 59.33	1.61 ± 56.36	−4.61 ± 57.91	0.93
12 months	4.07 ± 96.58	8.90 ± 91.33	7.91 ± 64.62	0.98
18 months	−28.96 ± 144.48	−20.59 ± 128.37	33.59 ± 118.59	0.30
Sclerostin				
6 months	−8.19 ± 32.64	5.35 ± 32.67	4.92 ± 35.41	0.23
12 months	2.10 ± 42.21	4.70 ± 34.06	16.02 ± 47.99	0.50
18 months	13.75 ± 47.56	7.44 ± 42.67	15.72 ± 41.30	0.77
Rs700518	AA	AG	GG	*P* value
CTX				
6 months	−37.42 ± 19.66	−18.13 ± 40.10	−8.59 ± 94.91	0.24
12 months	−17.31 ± 55.44	−0.50 ± 40.68	−6.03 ± 5.32	0.44
18 months	−9.75 ± 13.80	3.35 ± 9.64	41.47 ± 16.33[c,d]	**0.05**
Osteocalcin				
6 months	10.28 ± 56.76	−6.77 ± 58.06	−3.01 ± 56.26	0.53
12 months	20.56 ± 104.95	−4.41 ± 83.19	17.79 ± 71.62	0.49
18 months	−7.37 ± 112.06	−31.22 ± 148.00	12.40 ± 133.13	0.28
Sclerostin				
6 months	−6.60 ± 32.95	3.83 ± 33.13	0.52 ± 35.58	0.51
12 months	7.98 ± 43.83	1.77 ± 34.44	14.57 ± 48.09	0.54
18 months	23.58 ± 48.20	3.75 ± 41.71	15.09 ± 41.54	0.21

Means ± SD, Bold values are *p* values that are significant. CTX, [a]$p < 0.05$ GG vs CC, [b]$p < 0.05$ GG vs CG, [c]$p < 0.05$ GG vs. AA and [d]$p < 0.05$ GG vs. AG

CTX C-telopeptide

Discussion

Polymorphisms of *CYP19A1* are associated with differences in the risk for hormone-related disorders secondary to alterations in aromatase activity among the variants resulting in variable hormonal levels [25]. Accordingly, these polymorphisms were reported to influence BMD [5], fracture risk [12], skeletal response to postmenopausal hormone therapy [14], breast cancer risk and progression [23], disease-free survival in women with breast cancer on aromatase inhibitor therapy [29]; and aromatase inhibitor-associated bone loss [24], and changes in body composition in women with breast cancer [30]. Given the regulatory role of estradiol on the male skeleton [7–9] and body fat [31]; testosterone on lean mass [31]; and the effect of aromatase activity on circulating and local tissue androgen and estrogen concentrations, we investigated the effect of *CYP19A1* polymorphisms on bone and body composition response and susceptibility to side effects from testosterone therapy in men with hypogonadism.

Our results indicate that men with the GG genotype for both SNPs had the greatest increase in WBBMD, with no meaningful intergenotype changes in aBMD in other sites. However, there was a significant reduction in tibial bone size (total area, periosteal circumference) in rs1062033 GG genotype compared to no change in other genotypes. The difference in tibial bone size was only observed after adjusting for ALM. This influential role of muscle–bone interaction on the skeletal response to intervention was previously demonstrated by the positive correlation between changes in total hip BMD and thigh muscle volume in subjects undergoing lifestyle intervention [32]. Meanwhile, androgens contribute to bone size through its effect on increasing periosteal apposition and in increasing muscle

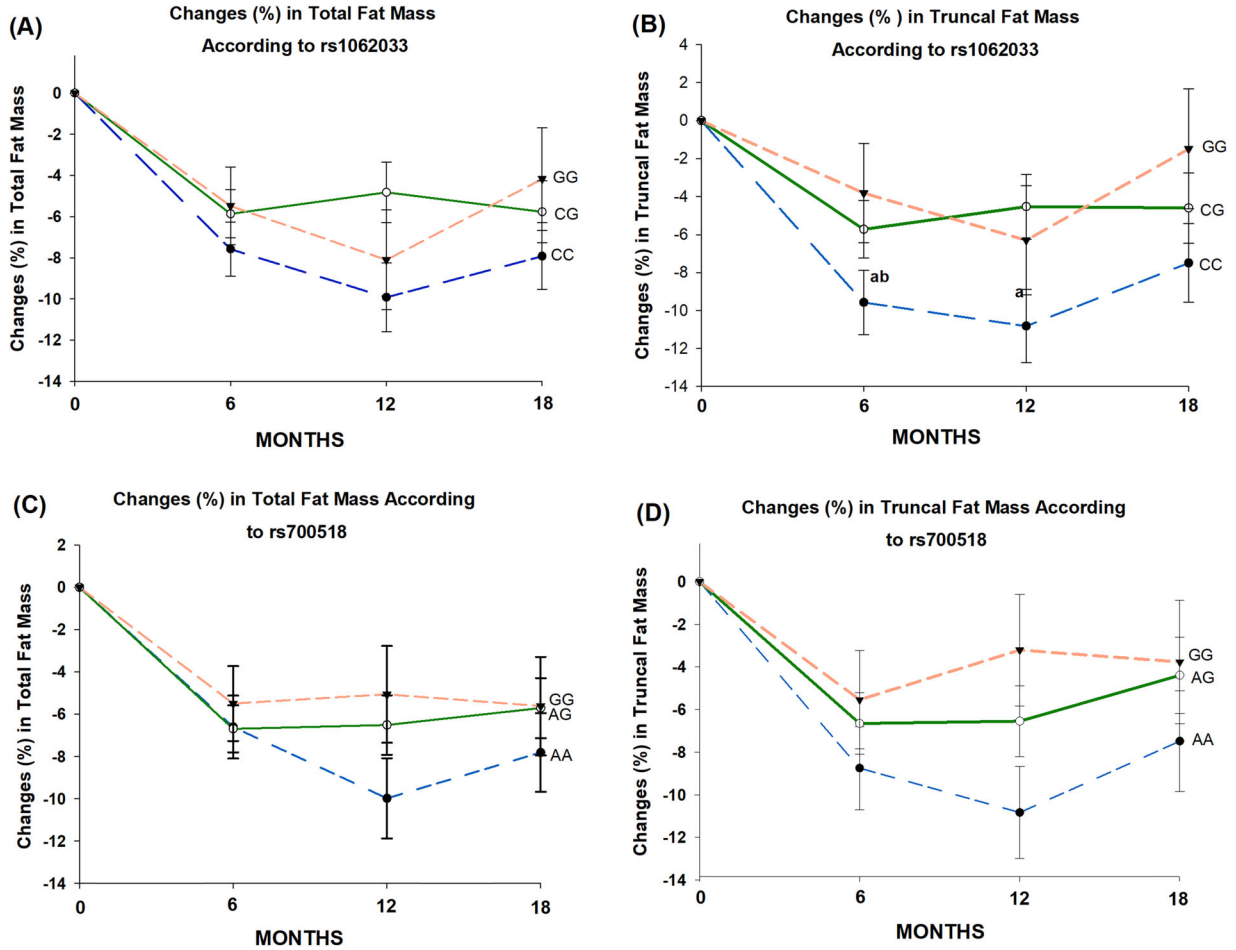

Fig. 2 Changes in Total and Truncal Body (%) With Testosterone Therapy According to rs1062033 and rs700518 Polymorphisms. Total fat mass decreased in all the genotypes of the rs1062033 at 6 months with no significant between-group differences. Total fat loss appears sustained at 12 months in the CC genotype with borderline significance compared to the other genotypes (overall $p = 0.08$); however, there is a regain in fat mass in those with the GG genotype with no significant intergenotype differences at 18 months (**a**). Truncal fat decreased in all the genotypes of the rs1062033, with the CC genotype having truncal fat low compared to the rest of the genotypes at 6 and 12 months (both $p = 0.03$) (**b**); however, there was a regain in truncal fat mass, most especially in the GG genotype, at 18 months Total fat mass decrease in all the genotypes of the rs700518 at 6 months with no significant between-group differences (**c**). Although fat loss continues in those with the AA genotype at 12 months, there was no significant intergenotype difference. A similar trend was observed in the changes in truncal fat among the genotypes of the rs700518 with trend for greater loss in the AA genotype at 12 months ($p = 0.08$) compared to the rest of the genotypes (panel D. All P values adjusted for age and body mass index. $^{a}p < 0.05$ CC vs. CG, $^{b}p < 0.05$ CC vs. GG

mass with resulting increased mechanical strain on bones. The former explains the bigger and wider bones in men compared to women [21] and, the latter for the larger bone size in the playing arms of tennis players [22]. Of note, men with the CC (rs1062033) and AA (rs700518) genotypes have trends for greater testosterone increase, which in turn likely contributed to the sustained increase in lean mass in both genotypes, perhaps ultimately influencing bone size maintenance in these men, not observed in men with the GG genotype. On the other hand, changes in bone turnover markers were not concordant with changes in BMD and bone geometry.

Fat mass loss, which is one of the reported benefits of testosterone therapy [33, 34], was not maintained among men with the greatest increase in circulating estradiol at 18 months, i.e. GG genotype for the rs1062033; a finding that seems to contradict with the report from Finkelstein et al. showing that reduction in estradiol levels is associated with increased fat mass in healthy men [31]. Moreover, the rest of the genotypes were able to maintain the reduction in fat mass at 18 months despite lesser degree of estradiol increase. However, circulating levels of estradiol and testosterone do not necessarily reflect tissue microenvironment concentrations of these hormones, because of local aromatase activity present in these tissues [35, 36]. In turn, this may explain for the lack of perfect alignment between the changes in bone and body composition with hormonal changes.

Higher mRNA transcripts were observed in the GG genotypes of both SNPs at 18 months indicating higher aromatase activity and estrogenic exposure perhaps accounting for the significant increase in WBBMD in the

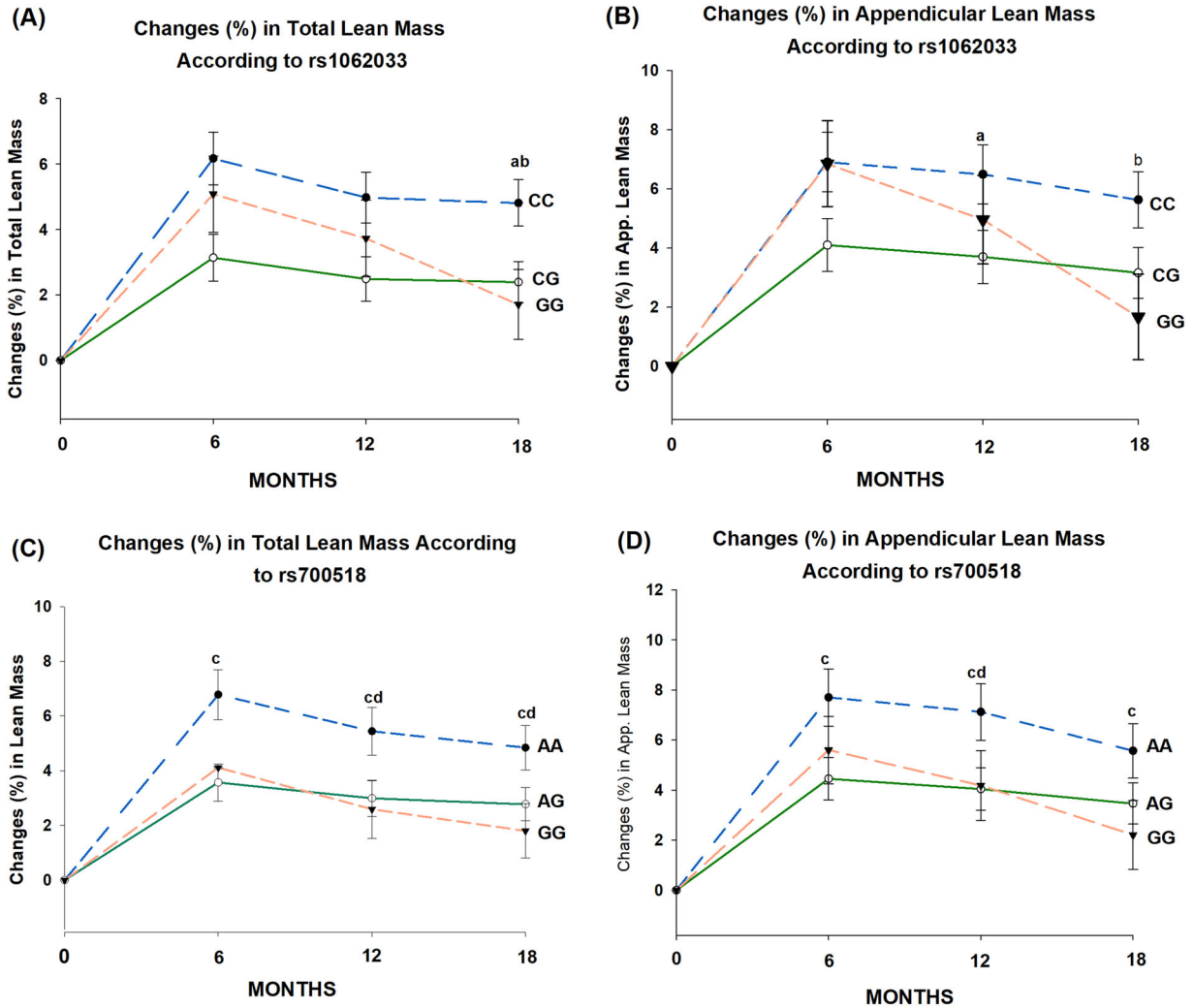

Fig. 3 Changes in Total and Appendicular lean mass (%) With Testosterone Therapy According to rs1062033 and rs700518 Polymorphisms. Total lean mass significantly increased in the CC genotype compared to the CG and GG genotypes at 18 months (**a**); and appendicular lean mass significantly increased in the CC compared to the CG genotype at 12 months and compared to the GG genotype at 18 months (**b**) in the rs1062033 polymorphism. There were increases in total lean mass in all the genotypes but significantly higher for the AA genotype of the rs700518 polymorphism compared to AG at 6 months, and compared to both AG and GG at 12 and 18 months (**c**). There were are also increases in appendicular lean mass in all the genotypes which was sustained to a certain degree in the AA and AG genotypes but not in the GG genotype (**d**). The increase in appendicular lean mass in the AA genotype was significantly higher compared to the AG genotype at 6 months, to both AG and GG genotypes at 12 months, and to the GG genotype at 18 months (**d**). All *P* values adjusted for age and body mass index. $^{a}p < 0.05$ CC vs. CG, $^{b}p < 0.05$ CC vs. GG, $^{c}p < 0.05$ AA vs. AG, $^{d}p < 0.05$ AA vs. GG

rs1062033 GG genotype. Riancho et al. showed higher adipose tissue *CYP19A1* mRNA levels in women with GG genotype of rs1062033 [13], and higher enzyme activity of the G relative to the C allele [5]. These investigators also showed that both SNPs belong to the same haplotype [13] and considers rs1062033 as the true regulatory SNP. We found a weak linkage of both SNPs in our sample and similarities in phenotypic responses to testosterone. Considering Riancho's findings [5], and given the location and the type of SNPs, our results concur that the rs1062033 as the likely biologically active SNP, may explain for the greater increase in WBBMD in rs700518 GG genotype despite the discordant changes in estradiol.

Limited studies have examined the effect of testosterone replacement on circulating *CYP19A1* mRNA levels. Vottero et al. reported increased expression in the peripheral blood leukocytes of men 3 days after testosterone administration [37], while He et al. showed downregulation of aromatase expression in the ovarian granular cells in women with polycystic ovarian syndrome given testosterone [38]. However, no genotypic analysis was done in these studies. Our study showed no significant difference in mRNA level at 18 months compared to baseline in the entire group, but inter-genotype differences in response to testosterone exist.

There were no significant differences in hematocrit changes among the genotypes in both SNPs. However, PSA

Table 6 Changes in estradiol and testosterone levels with testosterone therapy according to the rs1062033 and rs700518 polymorphisms of the *CYP19A1*

Rs1062033	CC	CG	GG	*P* value
Estradiol Δ (pmol/L)				
6 months	109.08 ± 87.51	71.53 ± 76.71	108.56 ± 83.63	0.13
12 months	62.18 ± 68.75	37.73 ± 54.96	88.85 ± 80.34[a]	**0.04**
18 months	67.17 ± 56.06	28.42 ± 65.00	87.13 ± 101.62[a]	**0.01**
Testosterone Δ (nmol/L)				
6 months	15.78 ± 11.84	11.20 ± 12.11	13.17 ± 8.52	0.23
12 months	11.55 ± 11.78	8.43 ± 10.55	16.78 ± 9.69[a]	**<0.05**
18 months	13.19 ± 10.36	7.63 ± 9.31	11.32 ± 9.42	0.06
Rs700518	AA	AG	GG	*P* value
Estradiol Δ (pmol/L)				
6 months	125.74 ± 95.64[b]	68.46 ± 68.22	109.61 ± 86.24	**0.02**
12 months	61.25 ± 69.10	50.93 ± 70.03	63.89 ± 61.51	0.75
18 months	72.57 ± 5.79	36.66 ± 73.59	70.92 ± 90.75	0.08
Testosterone Δ (nmol/L)				
6 months	18.11 ± 12.61[b]	10.40 ± 9.98	14.00 ± 12.00	**0.03**
12 months	11.41 ± 10.45	9.64 ± 11.00	14.55 ± 12.39	0.32
18 months	14.38 ± 10.69	8.52 ± 9.00	9.34 ± 10.22	0.06

Mean ± SD; Bold values are *p* values that are significant. [a]$p = 0.046$ GG vs CG, [b]$p = 0.046$ AA vs. AG; Δ: change

Table 7 Changes (%) in hematocrit and prostate-specific antigen (Psa) levels with testosterone therapy according to the rs1062033 and rs700518 polymorphisms of the *CYP19A1*

Rs1062033	CC	CG	GG	*P* value
Hematocrit				
6 months	10.29 ± 1.37	10.23 ± 1.30	11.30 ± 1.97	0.89
12 months	9.33 ± 1.52	9.72 ± 1.44	7.77 ± 2.30	0.77
18 months	9.13 ± 1.56	9.67 ± 1.45	11.56 ± 2.27	0.67
PSA				
6 months	54.78 ± 17.55	66.42 ± 15.88	68.43 ± 24.73	0.86
12 months	32.77 ± 10.96	43.70 ± 9.92	22.64 ± 14.99	0.48
18 months	56.89 ± 17.30	60.70 ± 15.65	85.07 ± 23.64	0.61
Rs700518	AA	AG	GG	*P* value
Hematocrit				
6 months	10.81 ± 1.54	10.58 ± 1.19	10.80 ± 1.90	0.99
12 months	9.27 ± 1.69	10.04 ± 1.33	7.64 ± 2.22	0.65
18 months	9.41 ± 1.73	9.71 ± 1.35	11.32 ± 2.21	0.77
PSA				
6 months	47.65 ± 19.25	64.79 ± 14.47	77.20 ± 24.55	0.62
12 months	21.93 ± 12.01	44.43 ± 9.03	31.53 ± 14.85	0.32
18 months	45.11 ± 18.71	58.55 ± 14.06	104.68 ± 23.14	0.12

Mean ± SD

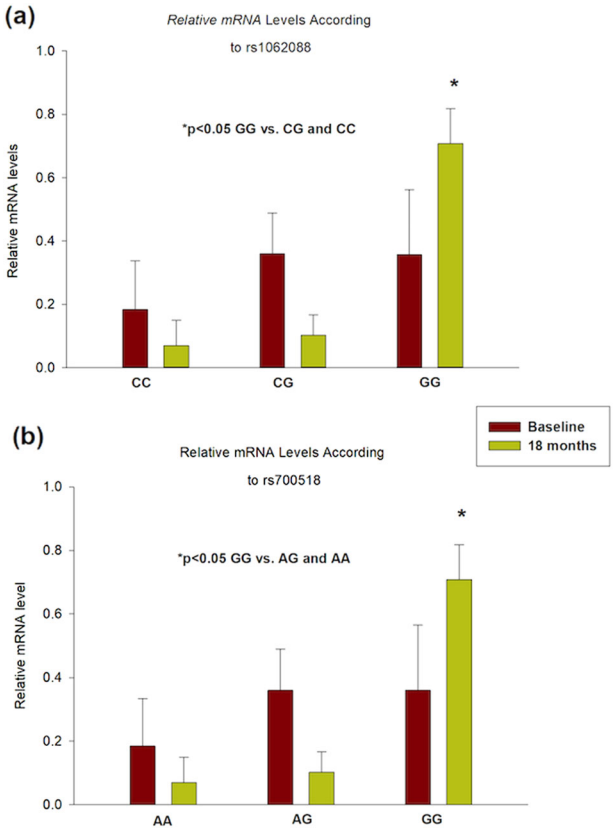

Fig. 4 mRNA levels significantly higher in the GG genotypes of both in both rs1062033 (**a**) and rs700518 (**b**) after 18 months of testosterone therapy. **p* value adjusted for baseline level

increase in subjects with the GG genotype for the rs700518 was significantly higher compared to the AG + AA genotypes combined at 18 months. The increased aromatase expression in the rs700518 GG genotype at 18 months would suggest increased conversion of testosterone to estradiol in tissues, including the prostate. Prostate is considered as an androgen-dependent organ, but estrogen has also been implicated in prostatic carcinogenesis [39]. Previous researchers have demonstrated that continuous estrogen exposure in the prostate can lead to inflammatory changes and ultimately to a pre-malignant state linking long-term estrogen exposure to prostate cancer development [40]. Hence, it is not surprising that certain polymorphisms in *CYP19A1* are associated with prostate cancer risk [41, 42], including the rs700518 [43]. Whether carriers of the GG genotype for the rs700518 are at risk for prostatic complications with testosterone therapy, remains undetermined. Our data in the limited number of subjects with prostate ultrasound at baseline and 18 months indicated no significant differences in prostate volume changes in both SNPs.

Our study has limitations. The small sample size and the relatively shorter duration of our study may limit our ability to detect significant differences in certain outcomes. In addition, we have a drop-out of 28% at the end of 18-months, which may induce bias in our findings. Regardless, there were no significant differences in baseline characteristics between those who continued and those who stopped study participation. Finally, the change in target testosterone level mandated by the FDA could have an effect on our results. Nevertheless, except for the paradoxically higher testosterone level at 6 months among those affected by the change, there were no significant differences in testosterone levels at 12 and 18 months between those affected and not affected by the change.

In conclusion, this the first interventional study investigating the effect of genetics on the response to testosterone therapy in hypogonadal men. Genetic profiling represents the most promising technique to predict response and the susceptibility to side-effects from certain medications, but, except for a few life-threatening diseases, is currently confined mostly to research. Our results suggest that differences in aromatase activity resulting from SNPs in *CYP19A1* may have long-term implications on clinical outcomes in hypogonadal men on testosterone therapy, highlighting the importance of genetic profiling even in common therapeutics, which in given time, may become a part of the standard of care.

Acknowledgements This study was supported by the resources at the New Mexico VA Health Care System in Albuquerque, NM, USA; the Biomedical Research of New Mexico, Albuquerque, NM, USA; the Michael E. DeBakey VA Medical Center, Houston, TX, USA and the Center for Translational Research in Inflammatory Diseases at the Michael E. DeBakey VA Medical Center, Houston, TX, Alkek Foundation, Houston, TX.

Author contributions R.A.-V. designed the study. G.C., L.E.A., I.Z.J., D.R., R.D., D.T.V., R.A.-V. conducted the study and collected the data. G.C., C.Q., D.T.V., R.A.-V. analyzed and interpreted the data. G.C., R.C., V.O.S., D.T.V., R.A.-V. drafted the paper. All the authors take responsibility for the paper content, the integrity of the data analysis and approval of the final version of the paper.

Funding This study was funded by VA Merit Review (5101 CX00042403) and 1 R01 HD093047-01.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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