Original Report

Role of the Androgen Receptor Gene CAG Repeat Polymorphism on the Sequence of Pubertal Events and Adiposity in Girls with High Dehydroepiandrosterone Sulfate Level

María Cecilia Lardone PhD [1], Andrea Castro MSc [1], Ana Pereira PhD [2], Camila Corvalán PhD [2], Eliana Ortíz BSc [1], Verónica Mericq MD [1,*]

[1] Institute of Maternal and Child Research, School of Medicine, University of Chile, Santiago, Chile
[2] Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile

A B S T R A C T

Study Objective: The androgen receptor (AR) harbors a variable repeat number of glutamine residues codified by (CAG)n, which seems to inversely affect AR transcriptional activity. We assessed whether (CAG)n affects the sequence of the androgen-sensitive pubertal events and body composition in prepubertal girls.
Design, Setting, Participants, and Interventions: Nested case-control study within the Growth and Obesity Cohort Study of 1196 low-middle income children (approximately 50% girls) from a university clinic in Santiago, Chile. Cases were girls with high dehydroepiandrosterone sulfate (DHEAS; >42 μg/dL; HD) at age 7.0 (±0.4) years (n = 58). On follow-up, 32 of them had thelarche (TB2) before the age of pubarche (PH2) and 26 had PH2 before the age of TB2. As controls, 107 age-matched girls with normal DHEAS (≤42 μg/dL; ND) were selected.
Main Outcome Measures: Methylation-weighted mean (CAG)n (mw[CAG]n) was calculated through X-chromosome methylation-sensitive enzyme restriction and polymerase chain reaction followed by automated capillary electrophoresis in peripheral blood DNA.
Results: Girls with HD and PH2 before the age of TB2 showed a trend to higher frequency (7/26, 26.9%) of mw(CAG)n <20 compared with ND girls (12/107; 11.2%; $P = .087$). Accordingly, a direct correlation between age of PH2 and mw(CAG)n was observed in HD ($r = 0.352$; $P = .007$) and in ND girls ($r = 0.207$; $P = .033$). Moreover, HD girls with mw(CAG)n less than 20 had lower waist circumference and waist/height ratio than HD girls with mw(CAG)n from 20 to less than 25 ($P = .027$ and $P = .012$, respectively) at age of DHEAS determination.
Conclusion: Our results suggest that a greater transcriptional activity of the AR, given by short number of CAG repeats, might favor the onset of pubarche and reduce central adiposity in prepubertal girls with HD.
Key Words: Androgen receptor CAG repeats, Body composition, DHEAS, Pubarche

Introduction

During normal pubertal development, only 10% of girls begin with pubic hair growth compared with 90% of girls whose first manifestation is thelarche. The growth of pubic hair or pubarche, depends on the rise of the adrenal androgen dehydroepiandrosterone sulfate (DHEAS) during a process called “adrenarche” around the age of 8 years in girls and 9 years in boys. These adrenal androgens act as precursors of the more potent androgens testosterone and dihydrotestosterone in peripheral tissues including the skin.

As a transcription factor, X-chromosome androgen receptor (AR) contains an amino terminal domain with transactivation function encoded in exon 1, which harbors a polymorphic repeat track of 9-36 residues of glutamine (Gln), encoded by contiguous CAG. In vitro studies have shown an inverse correlation between the number of CAG repeats ([CAG]n) and AR transcriptional activity.[1,2] Furthermore, it has been suggested an association between the length of the (CAG)n and pathologies sensitive to androgens such as prostate cancer,[3] breast cancer,[4] polycystic ovary syndrome,[5] and male infertility.[6] In this sense, a short number of (CAG)n has been associated with premature pubarche (PP) in Mediterranean girls with high or normal level of serum DHEAS.[7,8] These findings suggest that short CAG polymorphisms increases sensitivity of hair follicles to androgen as a possible pathogenic mechanism to PP. In addition, short (CAG)n polymorphisms might have a role in the heterogeneity of clinical manifestations in children with premature adrenarche (PA)[9] and nonclassical 21-hydroxylase deficiency.[10]

AR CAG repeat polymorphisms have also been associated with a greater body fat accumulation in healthy and PA prepubertal boys with long CAG repeats.[9,11] These findings suggest that AR CAG polymorphism might have a role in regulating androgen-mediated body composition before puberty. In this sense, our previous reports, on children enrolled in the Growth and Obesity Chilean Cohort Study (GOCS), showed that high levels of DHEAS at 7 years are associated with total and central adiposity, and to larger weight gain from age 4 years and beyond.[12,13]

Therefore, our aim was to assess whether the number of AR (CAG)n affects the sequence of androgen-sensitive pubertal events and body composition in girls with high

The authors indicate no conflicts of interest.
Presented at the 10th International Meeting of Pediatric Endocrinology, September 14 -17, 2017, Washington, DC.
* *Address correspondence to:* V. Mericq, MD, PO Box 226-3, Santiago, Chile; Phone: +56 2 4248280
E-mail address: vmericq@med.uchile.cl (V. Mericq).

1083-3188/$ - see front matter
https://doi.org/10.1016/j.jpag.2018.11.012

levels of serum DHEAS at 7 years (HD). We determined AR (CAG)n considering that 1 of the X-chromosomes is randomly inactivated by methylation and consequently not expressed (methylation-weighted CAG repeats [mw(CAG)n]).

Materials and Methods

Subjects

The study group is a subset sample of Chilean girls drawn from the GOCS. Briefly, this study was initiated in 2006 when children aged 2.6-4.0 years were recruited from public nursery schools of 6 counties in Santiago who met the following inclusion criteria: (1) singletons born in 2002-2003 with birth weight between 2500 and 4500 g; and (2) absence of physical (eg, skin burn), medical (eg, brain tumor), or endocrine diseases (eg, hyperthyroidism, hyperprolactinemia) that can alter the growth and/or onset of puberty. A complete description of the cohort is reported elsewhere.[12] The inclusion criteria for HD girls was a serum concentration of DHEAS greater than 42 μg/dL at the age of 7.0 (±0.4) years, and within these girls 2 groups were separated on the basis of age at which Tanner breast stage 2 (TB2) or age of appearance pubic hair stage 2 (PH2) occurred first. As controls, girls with serum DHEAS of 42 μg/dL or less (ND) at the age of 6.8 (±0.4) years for whom data regarding age of pubertal staging were also available. DHEAS analysis was previously performed in fasting venous blood samples using radioimmunoassay (intra- and interassay coefficient of variation (CV) were 3.5% and 5.1%, respectively) following the manufacturer's instructions (Diagnostic System Laboratories), and a DHEAS cutoff value of greater than 42 μg/dL was determined as the 75th percentile of GOCS distribution.[12]

The study protocol was approved by the institutional review board of the Institute of Nutrition and Food Technology of the University of Chile. Informed consent was obtained from parents or guardians of the children.

Clinical Assessment

Assessment of breast development using palpation and visual inspection according to the Tanner rating scale and the presence of sexual hair (axillary and pubic), yearly after recruitment and every 6 months after age approximately 7.0 years, was performed at the outpatient clinic of the Institute of Nutrition and Food Technology. Age of TB2 and PH2 were estimated according to the midpoint time between the age at last Tanner stage 1 and the age at the first TB2 visit, and between the age at the last visit with absence of pubic hair and the visit immediately after with the presence of pubic hair.[14]

Body Composition Indicators

Weight (in kilograms) and height (in centimeters) were measured with a portable electronic scale (Seca 770, Seca Ltd), and with a portable stadiometer (Harpenden 603; Holtain LTD) as previously described.[14] Sex- and age-adjusted body mass index *z*-score (BMI SDS) were calculated on the basis of the World Health Organization growth charts. Body fat percentage was estimated using bioelectrical impedanciometry (BIA) measurements (Tanita BC-418). Central adiposity was evaluated using waist circumference (WC) and waist to height ratio (W/H) using standardized procedures (the parameter distribution of this cohort is reported elsewhere[12]). Birth weight (in grams) was obtained from medical charts.

Genotyping and X-Chromosome Inactivation Analysis

X-chromosome inactivation analysis detects the random inactivation of one of the X-chromosomes occurred via DNA methylation during development,[15] which is associated with the methylation of *HpaII* sites close to CAG repeats in the AR gene.[16] Two separate reactions were prepared to investigate the methylated (transcriptionally inactive) and not methylated (transcriptionally active) allele. One microgram of DNA obtained from peripheral blood samples was incubated at 37°C overnight with 10 U of *HpaII* (New England Biolabs), and 1X NE Buffer 1 (New England Biolabs) in a final volume of 20 μL. A parallel reaction was carried out without the enzyme.

After enzymatic reactions, DNA was used to amplify the polymorphic 5′-terminal region containing the CAG repeat of the human AR. Briefly, 200 ng of digested or not digested DNA were amplified in a final volume of 15 μL containing 0.8 U of DyNAzime EXT DNA polymerase (Thermo Scientific), 450 nM of each primer, 2.5 mM of $MgCl_2$, 200 nM of each deoxynucleotide triphosphate (Invitrogen), 1X Mg^{2+}-free DyNAzime EXT Buffer (Thermo Scientific), and 8% DMSO (Thermo Scientific). Polymerase chain reaction conditions comprised an initial denaturation step at 95°C for 5 minutes, 35 cycles at 95°C for 45 seconds, 60°C for 45 seconds, 72°C for 1 minute, and a final extension step at 72°C for 10 minutes. The sequences of primers were previously reported (*gln* forward: 5′- TCC AGA ATC TGT TCC AGA GCG TGC-3′ labeled with NED™ and *gln* reverse: 5′-GCT GTG AAG GTT GCT GTT CCT CAT-3′).[17] Polymerase chain reaction fragment separation was performed using automated capillary electrophoresis in an ABI PRISM 310 Genetic Analyzer (Applied Biosystems), and analyzed with Gene mapper Analysis Software (version 3.2; Applied Biosystems). In each capillary electrophoresis, the fragment sizes were compared with fragments of known sizes obtained from human male DNA analyzed using automated sequencing (Macrogen Inc) published elsewhere.[18]

In heterozygotes, the percentage of inactivation of 1 allele was calculated using the formula (p1d/p1u)/(p1d/p1u + p2d/p2u) where p1 and p2 are the peak area of each allele in the digested (d) or undigested (u) amplification, as previously described.[9] Subsequently, the biallelic mean of mw(CAG)n was calculated by averaging the (CAG)n of each allele multiplied by its percentage of activation (100−percentage of inactivation). Skewing X-chromosome inactivation was determined when 80% or higher percentage was observed for either one of the alleles, as stated by Naumova et al,[19] and nonrandom inactivation was determined as >70% of inactivation.

Table 1
Age and Serum DHEAS Concentration in Girls

Variable	HD TB2 < PH2 (a)	HD PH2 < TB2 (b)	ND (c)
n	32	26	107
TB2, years	7.4 ± 0.5[b,c]	10.2 ± 0.8	9.7 ± 0.8
PH2, years	9.2 ± 0.7[c]	9.1 ± 0.9[c]	9.7 ± 0.9
DHEAS at 7 years, μg/dL	58.4 ± 18.1[c]	65.9 ± 18.7[c]	25.7 ± 9.2
TB2−PH2, years	−1.9 ± 0.9 (−3.1 to 0.4)[b,c]	1.2 ± 0.6 (0.3-2.5)[c]	0.15 ± 1.0 (−2.1 to 2.3)

DHEAS, dehydroepiandrosterone sulfate; HD, dehydroepiandrosterone sulfate >42 μg/dL at 7 years; ND, normal dehydroepiandrosterone sulfate at 7 years; PH2, age at pubarche; TB2, age at thelarche; TB2−PH2, difference between age of thelarche and pubarche.
Data are presented as mean ± SD or mean ± SD (range).
[a,b,c]Statistically significant difference with the corresponding group (a, b and c).

Statistical Analyses

Statistical analysis of the data was performed using SPSS software version 21 (IBM Corp) and GraphPad software PRISM 5.01. Analysis of variance with Bonferroni post hoc analysis and *t* test were used to analyze differences between groups, and the Spearman rank correlation coefficient (*r*) for correlation analysis. The difference between the arithmetic mean of (CAG)n and the mw(CAG)n was tested with paired samples *t* test. The (CAG)n and mw(CAG)n frequency distribution was evaluated using the χ^2 test followed by Bonferroni correction. A generalized linear model was used to evaluate interaction of DHEAS, age of thelarche, and age of pubarche over adiposity markers. $P < .05$ was considered statistically significant. From the mw(CAG)n distribution of the ND group, we defined short and long number of CAG repeat lengths as the values below the mean minus 1 SD or the value above mean plus 1 SD, respectively.

Results

Clinical Profile

A total of 58 girls with HD, of whom 32 had age at TB2 before the appearance of PH2 (TB2 < PH2) and 26 had age at PH2 before the age at TB2 (PH2 < TB2), were included. In the control group, we selected 107 girls with serum DHEAS of 42 μg/dL or less at the age of approximately 7 years and TB2 and PH2 after 8 years of age. Age of PH2 and TB2, the time between TB2 and PH2, and DHEAS concentration at age approximately 7 years in HD and ND girls are shown in Table 1. In the ND group, TB2 and PH2 occurred after the age of 8 years. In HD girls with TB2 < PH2, we observed a great proportion of thelarche occurring before 8 years and PH2 developed almost 2 years later. Conversely, when PH2 was the first secondary sex manifestation (only 2 HD girls with PH2 < TB2 had pubarche before 8 years of age), TB2 arose 1.2 years later, indicating a statistically significant difference with girls with TB2 < PH2 ($P = .017$; Table 1).

(CAG)n and mw(CAG)n Distribution and Correlation with Pubertal Events and Body Composition

One hundred sixty-five DNA samples were analyzed, of which 17/165 (114.4%) were homozygous for the polymorphism of CAG repeats. The (CAG)n and mw(CAG)n are presented in Table 2. The shortest and longest (CAG)n were 12 and 29 repeats, and the mean and median values of mw(CAG)n among the 3 groups were similar. A similar frequency of nonrandom inactivation was detected in girls with HD TB2 < PH2, HD PH2 < TB2, and controls (10/28 [35.7%]; 6/23 [26%]; and 36/97 [37.1], respectively).

Although the distribution of mw(CAG)n among the 3 groups was similar ($P = .445$; Fig. 1), after categorization of mw(CAG)n as short: mw(CAG)n less than 20; medium: mw(CAG)n from 20 to less than 25; and long: mw(CAG)n 25 or more, a trend to a higher frequency of short alleles in HD girls with PH2 < TB2 (7/26, 26.9%) compared with HD girls with TB2 < PH2 (6/32, 18.5%) and ND girls (12/107, 11.2%; $P = .087$) was observed (Fig. 2). Accordingly, a statistically significant direct correlation between age of PH2 and mw(CAG)n was observed in HD and in ND girls ($r = 0.352$ [$P = .007$] and $r = 0.207$ [$P = .033$], respectively). No correlation between mw(CAG)n and age of TB2 was observed.

Body composition parameters distribution in HD and ND girls at age approximately 7 years and at age of breast Tanner stage II are shown in Table 3. Similar to our previous reports in children enrolled in the GOCS,[12] in this subsample, we found that girls with HD had higher WC and W/H at the age of approximately 7 years compared with ND girls after adjusting for age of TB2 and PH2 ($P = .003$ and $P = .02$, respectively). Furthermore, girls with HD and PH2 < TB2 presented the largest WC and W/H (Table 3).

However, although HD girls at age approximately 7 years had increased WC and W/H, within these girls those with

Table 2
(CAG)n and (CAG)n Methylation-Weighted Biallelic Mean of Androgen Receptor Gene

	HD Girls TB2 < PH2	HD Girls PH2 < TB2	ND Girls
(CAG)n	21.8 ± 3.4 (22, *n = 64*)	21.6 ± 2.5 (22, *n = 52*)	22.1 ± 2.9 (22, *n = 214*)
mw(CAG)n	22.1 ± 2.5 (22.3)	21.6 ± 2.2 (21.6)	22.2 ± 2.2 (22.7)
Non-random inactivation, percentage of subjects	35.7	27.3	37.5

(CAG)n, variable number of CAG repeats; HD, dehydroepiandrosterone sulfate >42 μg/dL at 7 years; mw(CAG)n, biallelic mean of methylation-weighted of androgen receptor gene CAG repeats; ND, normal dehydroepiandrosterone sulfate at 7 years; PH2, age at pubarche; TB2, age at thelarche.
Values are presented as mean ± SD (median, *number of alleles*) except where otherwise noted.

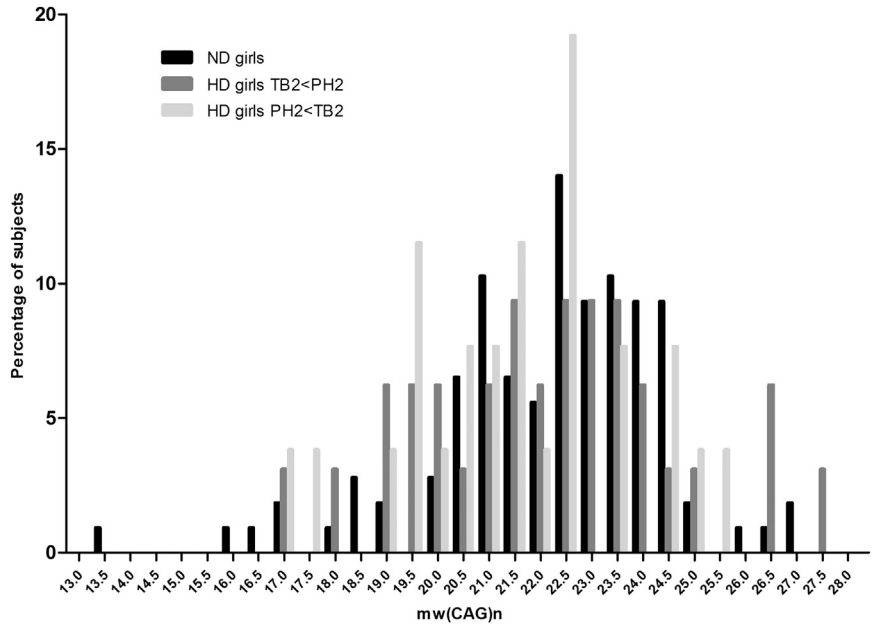

Fig. 1. Allele distribution of androgen receptor gene CAG repeats length in high dehydroepiandrosterone sulfate (DHEAS) and normal DHEAS (ND) girls. The frequency distribution of the biallelic mean of methylation-weighted of androgen receptor gene CAG repeats (mw[CAG]n) is displayed in girls with high concentrations of DHEAS (HD) grouped according to timing of pubarche (PH2) and thelarche (TB2) and in ND girls.

mw(CAG)n less than 20 (n = 13) had lower WC and W/H, and a trend to lower BMI SDS and percent fat mass than HD girls with mw(CAG)n from 20 to less than 25 (n = 39; Fig. 3). After adjusting for DHEAS concentrations, W/H maintained the statistical significance (P = .039). Moreover, HD girls with mw(CAG)n less than 20 had similar BMI SDS, percent fat mass, WC, and W/H than ND with the same mw(CAG)n repeats. On the other hand, at age of TB2 these differences of adiposity markers according to mw(CAG)n ranges disappear.

As might be expected, cases with TB2 < PH2 were taller at approximately 7 years and at the age of TB2 (Table 3). In all the studied girls, no correlations were observed between height at age approximately 7 years or height at age of TB2 and mw(CAG)n.

Discussion

In the present observational study we investigated the association between secondary sex characteristics appearance and body composition with AR (CAG)n polymorphisms in girls. We showed a positive correlation between age of PH2 and mw(CAG)n, in girls with HD and also in girls with ND, supporting a modulating effect of CAG repeats in the activity of AR independently of serum DHEAS concentrations. Vottero et al[8] described a decreased AR methylation in PP girls suggesting that the apparent derepression of AR expression in girls with PP can lead to hypersensitivity of hair follicles and thus to the premature development of pubic hair. In the present report, the potential AR transcriptional activity, in girls exposed to normal or high

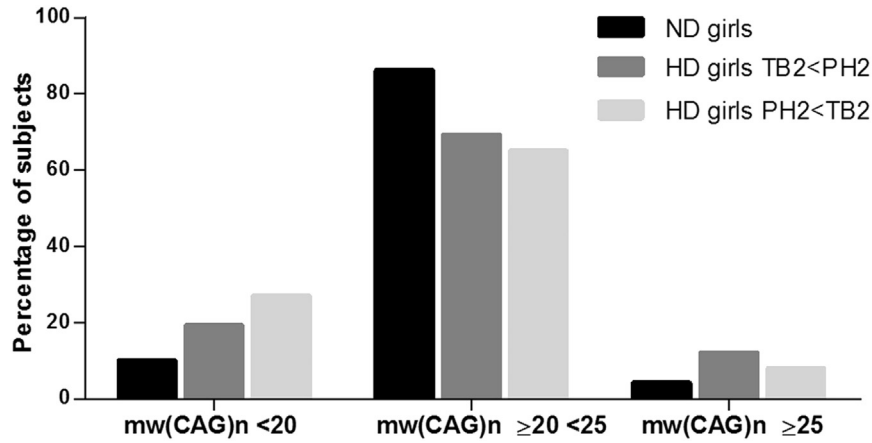

Fig. 2. Allele distribution comparison of biallelic mean of methylation-weighted of androgen receptor gene CAG repeats (mw[CAG]n) grouped in short (mw[CAG]n <20), medium (mw[CAG]n ≥20 <25), and long (mw[CAG]n ≥25) alleles in high dehydroepiandrosterone sulfate (DHEAS) and normal DHEAS (ND) girls. Girls with high concentrations of DHEAS (HD) are grouped according to timing of pubarche (PH2) and thelarche (TB2).

Table 3
Body Composition Parameters

	All HD Girls	HD girls TB2 < PH2	HD Girls PH2 < TB2	ND Girls	P*	P†	P‡	P‡
n	58	32	26	107				
Birth weight, kg	3.3 ± 0.4	3.2 ± 0.3	3.4 ± 0.3	3.4 ± 0.4	.165	.094	.240	1.000
BMI SDS at 7 years	1.0 ± 1.0	1.0 ± 0.9	1.2 ± 1.0	0.7 ± 1.0	.059	.755	1.000	.199
Body fat at 7 years, %	25.4 ± 4	25 ± 4	26 ± 4	24 ± 5	**.032**	.348	1.000	.223
Waist circumference at 7 years, cm	60.8 ± 7.0	60 ± 7	61 ± 8	58 ± 6	**.003**§	.105	1.000	**.044**§
Waist:height ratio at 7 years	0.5 ± 0.05	0.49 ± 0.05	0.51 ± 0.05	0.48 ± 0.04	**.047**§	1.000	.425	**.042**§
Height at 7 years, cm	121.9 ± 5.9	123.1 ± 5.6	120.3 ± 6	119.2 ± 4.7	**.002**§	**.001**§	.111	1.000
Height SDS at 7 years	0.3 ± 1.1	0.6 ± 1.1	0.07 ± 1.1	0.7 ± 0.8	.075	**.027**§	.138	1.000
BMI SDS at Tanner II	1.1 ± 1.1	1.0 ± 1.0	1.2 ± 1.2	0.7 ± 1.1	.118	.933	1.000	.390
Body fat at Tanner II, %	26.7 ± 4.9	26.0 ± 4.1	28.1 ± 6.3	26.8 ± 5.2	.909	1.000	.652	1.000
Waist circumference at Tanner II, cm	65.1 ± 9.2	61.9 ± 6.7	71.5 ± 10.3	67.6 ± 9.7	.145	**.011**§	**.004**§	.408
Waist:height ratio at Tanner II	0.49 ± 0.06	0.48 ± 0.05	0.50 ± 0.07	0.48 ± 0.06	.653	1.000	.501	.538
Height at Tanner II, cm	132.3 ± 8.8	128.3 ± 6.8	140.4 ± 6.7	138.7 ± 7	**.002**§	**.001**§	**.001**§	1.000
Height SDS at Tanner II	0.3 ± 1.3	0.6 ± 1.1	−0.25 ± 1.4	−0.8 ± 0.92	**.048**	**.008**§	**.037**§	1.000

BMI, body mass index; HD, dehydroepiandrosterone sulfate >42 μg/dL at 7 years; ND, normal dehydroepiandrosterone sulfate at 7 years; PH2, age at pubarche; SDS, z-score; TB2, age at thelarche.
Statistical test done using analysis of variance (ANOVA).
Statistically significant *P* values are shown in bold numbers.
* *P* values from comparison of all HD girls with ND girls.
† *P* values from comparison of girls TB2 < PH2 with ND.
‡ *P* values from comparison of girls PH2 < TB2 with girls with TB2 < PH2 and with ND, respectively.
§ Differences persist after adjusting for age at thelarche and pubarche using a generalized linear model.

DHEAS concentrations, seemed to favor the appearance of pubarche during pubertal development.

AR (CAG)n usually range between 12 and 35 with medians ranging from 21 to 23 in Hispanic and Caucasian populations.[20,21] Several in vitro studies have shown that variability in CAG repeat lengths might affect the AR transcriptional activity, and these results have shown a decrease in transcriptional activity as the number of CAG repeats increases, even within the normal range.[1,2,22] Therefore, repeats shorter than 20 are outside the median and would have a higher transcriptional potential.[1] In the same line of findings, Ibañez et al reported a higher frequency of biallelic mean (CAG)n of 20 or less in PP compared with healthy girls.[7] However, long (CAG)n repeats within the normal range ([CAG]n > 25) are associated with less frequency of PP and advanced gonadarche, and milder phenotypic presentation of androgen-sensitive manifestation in women with nonclassical 21-hydroxylase deficiency.[10]

The genotyping results of CAG polymorphism have been typically expressed as the biallelic mean without taking into account differential allelic expression due to the random inactivation of 1 of the X chromosomes in each cell.[23] Using methylation-weighted means, we found that mw(CAG)n less than 20 trends to be more frequent in girls with HD and PH2 < TB2 compared with HD girls with TB2 < PH2 and normal girls. This result suggests that the potential transcriptional of AR, because of a particular polymorphism in its transactivation region, would have an effect on the sequence of appearance of pubertal events. Studying a larger number of girls with these characteristics would be useful to corroborate this finding.

In the present study, no significant skewed or nonrandom inactivation toward the short or long allele was observed in agreement with others reports.[9,24–26] Nevertheless, the difference in the number of CAG repeats between cases and controls in those studies was stronger when the methylation-weighted mean was taken into account, instead of the biallelic mean.[9,24] In our experience the mw(CAG)n showed a stronger correlation with the age of pubarche than the biallelic mean in HD (r = 0.352 [P = .007] and r = 0.333 [P = .011]), and in ND girls (r = 0.207 [P = .033] and r = 0.165 [P = .134]). These results suggest that the determination of the degree of inactivation gives a more accurate result of the contribution of the CAG polymorphism in the activity of the AR.

Additionally, we have corroborated in this subsample of girls what we previously described in the larger complete GOCS cohort: children with HD are more adipose.[12,27] Further, our results show that at approximately 7 years of age, HD girls who carried shorter CAG repeat lengths (mw [CAG]n <20), showed lower central adiposity, overweighting that of DHEAS concentrations, being similar to girls with ND with the same CAG length polymorphisms. However, at the age of TB2 no differences in body composition were observed, probably because of the higher levels of circulating androgen. Similar results were reported by Lappalainen et al[9] in children with PA, although no follow-up was performed to evaluate body composition during pubertal development. Follow-up of the GOCS cohort is necessary to address prospectively whether these interrelationships of AR CAG repeats and body composition are ameliorated or persist with higher androgen concentrations.

Another important event during pubertal development is the increase in growth velocity and finally attainment of adult height. In our girls no trends or associations between height and AR (CAG)n polymorphisms were observed at age approximately 7 years, or at an early pubertal stage (Tanner II). However, girls with TB2 < PH2 were taller independently of CAG repeat length, which might be explained by the primordial effect of low-dose estrogens increasing growth velocity,[28] which correlates with high levels of serum estradiol equivalents in girls with TB2 before 8 years, as we previously described.[27]

Our study is not exempt of limitations. The group of girls with HD and TB2 < PH2 might be biased toward an earlier

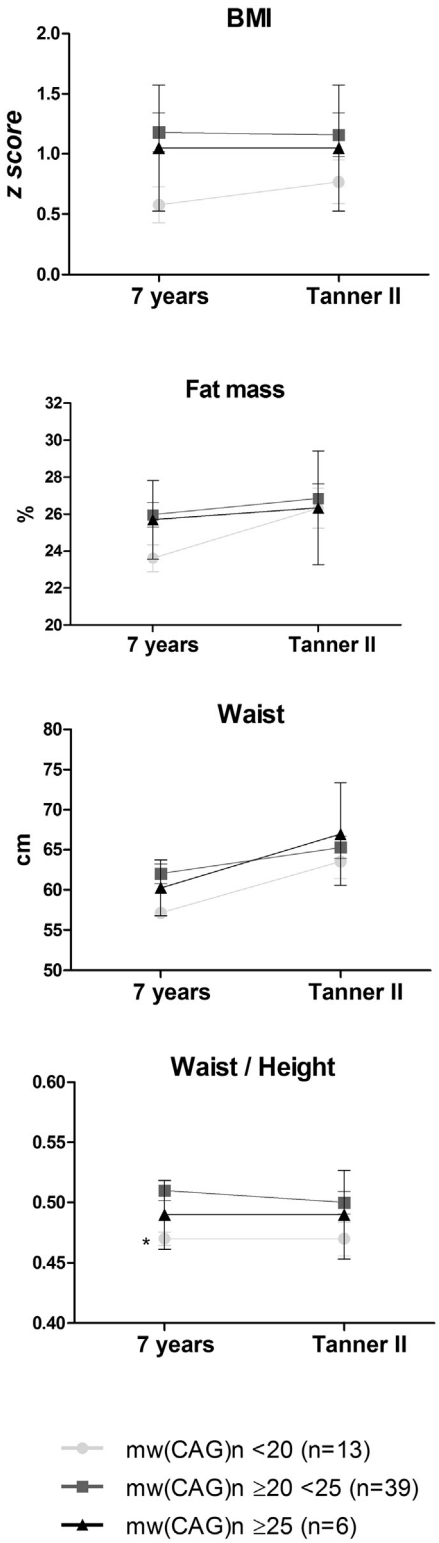

Fig. 3. Adiposity markers in girls with high dehydroepiandrosterone sulfate (DHEAS) at the age of 7 years and at the age of breast stage Tanner II according to biallelic mean of methylation-weighted of androgen receptor gene CAG short (mw[CAG]n <20), medium (mw[CAG]n ≤20 <25), and long (mw[CAG]n ≥25) repeat length. * $P < .05$ compared with girls with mw(CAG)n from 20 to less than 25 at the same pubertal time after adjusting for DHEAS concentrations. The figures and the lines represent mean ± standard error of the mean, respectively. BMI, body mass index.

age of thelarche (appearance of breast bud before 8 years of age) because in HD girls there is a 4 times risk for this event.[29] However, the occurrence of precocious pubarche is much less common among these girls (7.8%),[29] therefore in the group of HD girls with PH2 < TB2, pubarche before 8 years would be under-represented. Another point is that our observational study lacks a mechanistic approach to validate our findings, however they are strengthened by the nested case-control design within a cohort of approximately 500 girls with the same inclusion criteria. Third, body composition assessment was performed using bioelectrical impedance and anthropometry, which has an excellent correlation with lean mass but not as high with body fat. Nevertheless, BIA offers some advantages: is not invasive, needs little time of assessment, and has a low cost, which makes it very suitable for large-scale studies. Additionally, in our experience, BIA has a strong correlation with the measurement of skinfold thickness ($r = 0.9$). Dual-energy X-ray absorptiometry (DXA) and magnetic resonance imaging offer a greater accuracy in determining body fat and distribution. However, the use of these techniques is limited in a large number of subjects because of their cost, is time-consuming, and has some radiation exposure.

In conclusion, a clinical implication of our results is that the appearance of pubarche as a first event during pubertal development, in girls exposed to normal or high DHEAS concentrations, appears favored by the potential AR transcriptional activity. Second, the AR transcriptional activity does not seem related to age at thelarche onset. However, we and others have previously determined that obesity and DHEAS are positively associated in prepubertal children, however, the causative effect of this association remains to be determined.[9,12,30] Moreover, there is substantial evidence showing that AR CAG polymorphisms modulate androgen action within fat accumulation in young and adult men.[11,31,32] Taking this into consideration and that sex differences in body composition before puberty are moderate, we speculate that a more sensitive AR together with elevated androgens for prepubertal age, favors a lower accumulation of fat in these girls.

Acknowledgments

This work was supported by a 2015 Grant for Research Projects of the Chilean Society of Endocrinology and Diabetes to M.C.L. (grant number 2015-05), and by the National Fund for Scientific and Technological Development of Chile to V.M. (grant number 1140447).

References

1. Buchanan G: Structural and functional consequences of glutamine tract variation in the androgen receptor. Hum Mol Genet 2004; 16:13
2. Chamberlain NL, Driver ED, Miesfeld RL: The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acids Res 1994; 15:22
3. Hardy DO, Scher HI, Bogenreider T, et al: Androgen receptor CAG repeat lengths in prostate cancer: correlation with age of onset. J Clin Endocrinol Metab 1996; 12:81
4. Mao Q, Qiu M, Dong G, et al: CAG repeat polymorphisms in the androgen receptor and breast cancer risk in women: a meta-analysis of 17 studies. Onco Targets Ther 2015; 8:2111
5. Zhang T, Liang W, Fang M, et al: Association of the CAG repeat polymorphisms in androgen receptor gene with polycystic ovary syndrome: a systemic review and meta-analysis. Gene 2013; 2:524
6. Xiao F, Lan A, Lin Z, et al: Impact of CAG repeat length in the androgen receptor gene on male infertility - a meta-analysis. Reprod Biomed Online 2016; 1:33

7. Ibañez L, Ong KK, Mongan N, et al: Androgen receptor gene CAG repeat polymorphism in the development of ovarian hyperandrogenism. J Clin Endocrinol Metab 2003; 7:88
8. Vottero A, Capelletti M, Giuliodori S, et al: Decreased androgen receptor gene methylation in premature pubarche: a novel pathogenetic mechanism? J Clin Endocrinol Metab 2006; 3:91
9. Lappalainen S, Utriainen P, Kuulasmaa T, et al: Androgen receptor gene CAG repeat polymorphism and X-chromosome inactivation in children with premature adrenarche. J Clin Endocrinol Metab 2008; 4:93
10. Ben-Shachar S, Ayalon I, Reznik-Wolf H, et al: Androgen receptor CAG repeat length in relation to phenotype among females with nonclassical 21-hydroxylase deficiency. Horm Metab Res 2015; 7:47
11. Mouritsen A, Hagen CP, Sorensen K, et al: Androgen receptor CAG repeat length is associated with body fat and serum SHBG in boys: a prospective cohort study. J Clin Endocrinol Metab 2013; 3:98
12. Corvalan C, Uauy R, Mericq V: Obesity is positively associated with dehydroepiandrosterone sulfate concentrations at 7 y in Chilean children of normal birth weight. Am J Clin Nutr 2013; 2:97
13. Mericq V, Pereira A, Uauy R, et al: Early BMI gain and later height growth predicts higher DHEAS concentrations in 7-year-old Chilean children. Horm Res Paediatr 2017; 1:87
14. Pereira A, Garmendia ML, Gonzalez D, et al: Breast bud detection: a validation study in the Chilean growth obesity cohort study. BMC Womens Health 2014; 14:96
15. Zeng SM, Yankowitz J: X-inactivation patterns in human embryonic and extra-embryonic tissues. Placenta 2003; 2-3:24
16. Allen RC, Zoghbi HY, Moseley AB, et al: Methylation of HpaII and HhaI sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. Am J Hum Genet 1992; 6:51
17. Vottero A, Stratakis CA, Ghizzoni L, et al: Androgen receptor-mediated hypersensitivity to androgens in women with nonhyperandrogenic hirsutism: skewing of X-chromosome inactivation. J Clin Endocrinol Metab 1999; 3:84
18. Castro-Nallar E, Bacallao K, Parada-Bustamante A, et al: Androgen receptor gene CAG and GGN repeat polymorphisms in Chilean men with primary severe spermatogenic failure. J Androl 2010; 6:31
19. Naumova AK, Plenge RM, Bird LM, et al: Heritability of X chromosome–inactivation phenotype in a large family. Am J Hum Genet 1996; 6:58
20. Ackerman CM, Lowe LP, Lee H, et al: Ethnic variation in allele distribution of the androgen receptor (AR) (CAG)n repeat. J Androl 2012; 2:33
21. Esteban E, Rodon N, Via M, et al: Androgen receptor CAG and GGC polymorphisms in Mediterraneans: repeat dynamics and population relationships. J Hum Genet 2006; 2:51
22. Beilin J, Ball EM, Favaloro JM, et al: Effect of the androgen receptor CAG repeat polymorphism on transcriptional activity: specificity in prostate and non-prostate cell lines. J Mol Endocrinol 2000; 1:25
23. Migeon BR: Why females are mosaics, X-chromosome inactivation, and sex differences in disease. Gend Med 2007; 2:4
24. Hickey T, Chandy A, Norman RJ: The androgen receptor CAG repeat polymorphism and X-chromosome inactivation in Australian Caucasian women with infertility related to polycystic ovary syndrome. J Clin Endocrinol Metab 2002; 1:87
25. Calvo RM, Asuncion M, Sancho J, et al: The role of the CAG repeat polymorphism in the androgen receptor gene and of skewed X-chromosome inactivation, in the pathogenesis of hirsutism. J Clin Endocrinol Metab 2000; 4:85
26. Kang MJ, Lee JS, Kim HY, et al: Contributions of CAG repeat length in the androgen receptor gene and androgen profiles to premature pubarche in Korean girls. Endocr J 2017; 1:64
27. Pereira A, Corvalan C, Uauy R, et al: Ultrasensitive estrogen levels at 7 years of age predict earlier thelarche: evidence from girls of the growth and obesity Chilean cohort. Eur J Endocrinol 2015; 6:173
28. Juul A: The effects of oestrogens on linear bone growth. Hum Reprod Update 2001; 3:7
29. Pereira A, Iniguez G, Corvalan C, et al: High DHEAS is associated with earlier pubertal events in girls but not in boys. J Endocr Soc 2017; 7:1
30. Reinehr T, de Sousa G, Roth CL, et al: Androgens before and after weight loss in obese children. J Clin Endocrinol Metab 2005; 10:90
31. Ponce-Gonzalez JG, Rodriguez-Garcia L, Losa-Reyna J, et al: Androgen receptor gene polymorphism influence fat accumulation: a longitudinal study from adolescence to adult age. Scand J Med Sci Sports 2016; 11:26
32. Zitzmann M, Gromoll J, von Eckardstein A, et al: The CAG repeat polymorphism in the androgen receptor gene modulates body fat mass and serum concentrations of leptin and insulin in men. Diabetologia 2003; 1:46