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RESEARCH LETTER

Role of androgen receptor CAG repeat polymorphism length in hypothalamic progesterone sensitivity in hyperandrogenic adolescent girls

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Introduction

Polycystic ovary syndrome (PCOS) is characterized by oligo/anovulation, hyperandrogenism, and in many cases, polycystic ovaries. Women with PCOS often exhibit persistently increased gonadotropin-releasing hormone (GnRH) pulse frequency compared to normal cycling women, contributing to enhanced luteinizing hormone (LH) secretion and increased androgen production [1]. Administration of progesterone (P) decreases GnRH pulse frequency in normal women, but the GnRH pulse generator in women with PCOS is relatively insensitive to P negative feedback [2]. Treatment with the androgen receptor (AR) antagonist flutamide restores GnRH pulse generator sensitivity to P inhibition in women with PCOS [3]. Hyperandrogenemia in adolescence can represent a forerunner of adult PCOS, though the etiology of PCOS remains unclear. As a group, adolescent girls with HA display impaired GnRH pulse generator sensitivity to progesterone inhibition (i.e., reduced P-sensitivity). However, P-sensitivity is varied in HA girls, despite comparable levels of plasma-free testosterone (T) [4].

The AR gene contains a polymorphic trinucleotide (CAG) repeat sequence located in the N-terminal transactivation domain of the AR [5], and the number of CAG repeats influences AR activity. In vitro, expression of AR alleles with shorter CAG repeat lengths is associated with increased AR activity [6]. We measured AR CAG repeat length in a subgroup of subjects previously shown to have varied GnRH pulse generator sensitivity to P-mediated slowing [4], hypothesizing that shorter AR CAG repeat length would be associated with reduced P-sensitivity in HA girls.

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Materials and methods

The Institutional Review Board at the University of Virginia Health System approved the study. Informed assent and consent were obtained from all study volunteers and their parents. Data regarding GnRH sensitivity to P from 22 normal control (NC) and 24 hyperandrogenic (HA) girls have been reported previously [4]. Of this group, we selected 13 girls from the NCs (no evidence of hyperandrogenism) and 12 adolescent girls with HA, classified on the basis of biochemical (free T $>$ 2.5 SD values above the mean for normal weight control subjects of the same Tanner stage of breast development) and/or clinical hyperandrogenism (hirsutism) [4]. Six HA subjects had P sensitivities (sensitivity of the GnRH pulse generator to suppression by P) within the range of NCs, and six were insensitive to progesterone. In these subgroups, baseline hormone levels (total T, sex hormone-binding globulin, free T, DHEAS, androstenedione, estrone, estradiol, P, LH, FSH, and fasting insulin), age, years post menarche, and body mass index were all within the ranges previously reported. All samples were analyzed as described previously [4].

DNA was isolated from peripheral blood leukocytes using a Biorad AquaPure Genomic DNA kit (Biorad, Hercules, CA, USA). The PCR primers used to amplify the AR CAG repeat segment were: 5′-TCCAGAATCTGTTCCAGAGCG TCC-3′ (sense) and 5′-GCTGTGAAGGTTGCTGTTCC

TCAT-3′ (anti-sense) (Integrated DNA Technologies, Inc., Coralville, IA, USA). Fragment analysis was performed with GeneScan software (Applied Biosystems, Foster City, CA, USA).

Data are presented as the mean ± standard deviation for the mean unless indicated. Hypothesis tests were two-sided and were conducted at the 0.05 level of significance. LH pulses and P-sensitivity were defined as described previously [4]. Comparisons employed nonparametric statistical tests based on ranks of observations requiring no assumptions about the underlying distribution of data. Wilcoxon rank sum tests were used for pairwise comparisons, while Kruskal–Wallis tests were used for comparisons among three groups. Spearman rank correlation was used to evaluate the relationship between P-sensitivity and CAG repeats in all subjects.

Results

Hypothalamic progesterone sensitivities in 13 NC versus 12 HA girls were compared (Fig. 1) to data observed in a larger group of NC adolescents previously evaluated for hypothalamic P-sensitivity [4]. Of the 12 HA girls included in this study, 6 fell within the range of responses observed in NC adolescent girls, while 6 were relatively insensitive to P suppression (Fig. 1). In this study, hypothalamic P-sensitivity (i.e., the slope of percent change in LH pulses/11 h as a function of day 7 P concentration in ng/ml) was 20.7 ± 9 for Tanner 1-2 NC girls and 9.6 ± 7 for Tanner 3–5 NC girls. This was compared to 7.8 ± 3 and 1.6 ± 1 for Tanner 3–5 HA P-sensitive and HA P-insensitive girls, respectively. Free testosterone values were elevated in HA girls as a group compared to Tanner 3–5 NCs (10.3 ± 4 vs. 3.6 ± 2 pg/ml), but free T levels in HA P-sensitive versus P-insensitive girls (10.6 ± 3 vs. 10.0 ± 6 pg/ml) were similar.

We measured AR CAG repeat length in these 25 girls to determine whether there was a correlation with hypothalamic P-sensitivity. No significant differences of AR CAG repeat length were observed among groups. The NC group had a CAG repeat biallelic mean of 21.5 ± 2, compared to 20.6 ± 3 ($P = 0.52$) and 21.2 ± 2 ($P = 0.66$) for HA P-sensitive and P-insensitive groups, respectively (Table 1). CAG repeat length in individual short and long AR alleles among the groups were also similar (Table 1). In addition, there was no simple correlation between P-sensitivity (slope) and CAG repeat number, either when including all girls (HA and controls) or when restricting analysis to HA girls only. Simultaneously adjusting for free T differences did not alter these correlation findings.

Discussion

The clinical significance of variable hypothalamic P-sensitivity in adolescent girls with HA remains unclear, but may explain why not all HA girls go on to develop

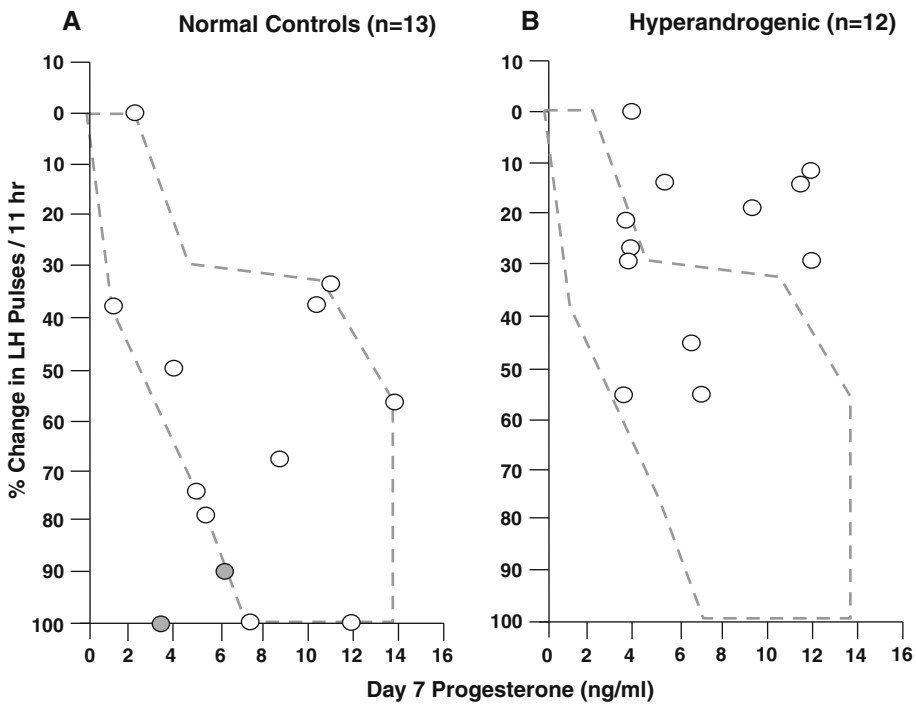

Fig. 1 Suppression of LH pulses after 7 days of estradiol and progesterone in NC and HA girls. Data are shown as percent reduction in LH pulses per 11 h as a function of day 7 P concentration. *Circles* represent NC (*left*) and HA (*right*) girls in which AR CAG repeat data was obtained. *Closed circles* represent Tanner 1-2 girls; *white circles* represent Tanner 3–5 girls. The area outlined by the *dashed line* represents the range of responses in Tanner 3–5 NC girls. (Data are redrawn from Blank et al. with permission [4]; *Copyright 2009, The Endocrine Society*)

Table 1 AR CAG repeat lengths in HA and NC girls

	Short allele	Long allele	Biallelic mean
NC ($n = 13$)	19.5 ± 1.2 (0.6)	23.6 ± 2.1 (0.6)	21.5 ± 1.6 (0.4)
HA P-sensitive ($n = 6$)	18.2 ± 3.7 (1.5)	23.0 ± 3.1 (1.3)	20.6 ± 3.2 (1.3)
HA P-insensitive ($n = 6$)	18.2 ± 3.4 (1.4)	24.2 ± 3.1 (1.2)	21.2 ± 1.7 (0.7)

Data are presented as mean ± SD (SEM)

abnormal regulation of LH secretion. Specifically, HA girls who demonstrate abnormal P suppression of GnRH pulse secretion may be more likely to progress to adult PCOS compared to those with normal feedback sensitivity. Although, androgen excess appears to decrease hypothalamic P-sensitivity in adult PCOS, differences in free testosterone concentrations do not appear to explain variable P-sensitivity in HA adolescents.

The influence of CAG repeat length on androgen action in women is unclear. Girls with precocious pubarche, associated with increased T, acne, and hirsutism, display shorter CAG repeat lengths compared to controls [7]. While some groups have found no association between CAG repeat length and PCOS [8, 9], others suggest a link between shorter CAG repeat lengths and symptoms of PCOS in women with normal T levels [10, 11]. We hypothesized that HA girls who are resistant to GnRH suppression by P would have shorter AR CAG repeat lengths compared to girls who maintain sensitivity to suppression, with shorter repeat lengths leading to increased AR activity. However, we found no evidence of an association between CAG repeat length (biallelic mean, long allele, or short allele) and hypothalamic P-sensitivity, suggesting CAG repeat length alone is not a strong determinant of hypothalamic P-sensitivity.

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Conflict of interest All authors have nothing to disclose.

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