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Association of the –243 A→G Polymorphism of the Glutamate Decarboxylase 2 (*GAD2*) Gene with Obesity in Girls with Premature Pubarche

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Abstract

Objective—To test the a priori hypothesis that the frequency of a single nucleotide polymorphism (SNP) located in the promoter region of the glutamate decarboxylase 2 (*GAD2*) gene (–243A→G) would be over-represented among children with higher BMI values.

Design—Genotype-phenotype correlation study.

Setting—University-based pediatric endocrinology practice.

Patients—Eighty-seven girls with PP and 70 adolescent girls with hyperandrogenism (HA).

Intervention(s)—Blood was obtained for genotype analysis, glucose measurement, and hormone (androstenedione, insulin, 17-hydroxyprogesterone, and testosterone) determinations.

Main Outcome Measure(s)—Frequency of this SNP in the *GAD2* gene and correlation of this SNP with BMI and hormone concentrations.

Results—Among the girls followed longitudinally, the presence of one or more G alleles was associated with increased BMI at both initial and recent visits and greater BMI Z-score at the initial visit. No associations were found between androgen concentrations and the G allele variant.

Conclusions—Similar to the findings among French children, this SNP in the *GAD2* gene was associated with increased BMI in late childhood and adolescence in this population of girls from Western Pennsylvania. Additional prospective studies are crucial to replicate our findings. Verification of our findings will encourage the use of lifestyle interventions for young girls who carry the G allele.

Keywords

Premature Pubarche; Polycystic Ovary Syndrome; Premature Adrenarche; Obesity; Glutamate Decarboxylase-2 (*GAD2*)

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Introduction

Premature pubarche (PP) is defined as the development of pubic hair prior to age 8 years in girls. One common cause of PP is premature adrenarche (PA) which is premature adrenal pubertal maturation. During the peri-pubertal years, some girls with a history of PP develop clinical features consistent with polycystic ovary syndrome (PCOS). Adolescent girls with symptoms of PCOS often manifest metabolic abnormalities comparable to those identified in women with PCOS such as insulin resistance, hyperinsulinemia, lower first-phase insulin secretion, LH hypersecretion, dyslipidemia, impaired glucose tolerance (IGT), and type 2 diabetes mellitus (1–3). Laboratory features associated with hyperinsulinemia such as decreased SHBG concentrations, decreased IGFBP1 concentrations, and increased IGF1 concentrations have been found among adolescent girls with PCOS (4–6). Increased carotid medial thickness, a risk marker for atherosclerosis, was detected in 18–22 year old women with PCOS (7). PCOS also adversely affects quality of life for adolescent girls (8). Thus, many of the potential deleterious consequences of PCOS are already evident in adolescent girls with PCOS.

Approximately 35% of mothers and 40% of sisters of women with PCOS also have PCOS (9). The concordance rates for PCOS in twin pairs were 60% among dizygotic twin pairs and 74% among monozygotic twin pairs (10). Thus, genetic factors contribute to the development of PCOS. Although no single specific “PCOS gene” has been identified, case-control association studies have shown that several genes are associated with the development of PCOS (11,12). However, small sample sizes, differences in allele frequencies, and epigenetic interactions have hindered the reproducibility of association studies (13). Available data implicate gene-environment and gene-gene interactions as influences on the phenotypic manifestations of PCOS (14,15).

Identification of the factors associated with an increased risk to develop PCOS will enable timely therapeutic interventions to decrease the morbidities associated with PCOS (16–18). To establish these risk factors, the relationship between PP and PCOS has been scrutinized in several populations. At multiple timepoints from presentation with PP to adolescence, lean Catalunyan girls are found to have decreased insulin sensitivity, hyperinsulinemia, dyslipidemia, increased central fat distribution, and increased plasminogen activator-inhibitor type 1 (PAI-1) activity (19–21). African-American and Caribbean-Hispanic girls with a prior history of PP are also reported to have an increased incidence of insulin resistance and hyperinsulinemia (22,23).

Some girls with PP and women with PCOS are obese. Greater post-natal weight gain during childhood has been associated with higher DHEAS concentrations and urinary androgen excretion (24,25). Obesity, prematurity, and SGA status were associated with premature pubarche in a small group of Australian children (26). Peripubertal obesity has been associated with hyperandrogenemia and hyperinsulinemia (27,28). Thus, excessive peri-pubertal weight gain may increase the risk for progression from PP to PCOS (29). Genome-wide scans for obesity genes have repeatedly detected linkage with a locus at chromosome 10p11.22–23 (30). One candidate obesity gene located within this region is the glutamate decarboxylase 2 (*GAD2*) gene. This gene codes for the 65-kDa subunit of glutamic acid decarboxylase (*GAD65*) which catalyzes the formation of γ -aminobutyric acid (GABA) from glutamate. In the paraventricular nucleus, GABA interacts with neuropeptide Y (NPY) to stimulate food intake. It has also been suggested that in pancreatic islets, GABA modulates hormone secretion (31). *GAD65* is a target of autoantibodies identified in individuals who later develop type 1 diabetes mellitus.

A single nucleotide polymorphism (SNP) located in the promoter region of the GAD2 gene, (−243A→G), rs2236418, has been associated with morbid obesity in French adults and severe childhood obesity in French children (32,33). Family-based genetic analyses showed association of obesity with two other SNPs, 61450C→A and 83897T→A, also located in the GAD2 gene (32). One functional analysis using a luciferase reporter assay showed that the (−243A→G) SNP increased transcriptional activity compared to the wild type allele; one consequence of this increased promoter activity could be increased GABA production with potential effects on feeding behavior and insulin secretion (32). Nevertheless, other investigators have been unable to replicate this association (34). To determine whether this SNP was associated with PP or predicted outcome in patients with PP, we ascertained the frequency of this SNP in our patient population.

Materials and Methods

Subjects

Eighty-seven girls were referred to the Children's Hospital of Pittsburgh for the evaluation of premature development of pubic hair (PP). By definition, pubic hair had developed prior to age 8 years in these girls. Among these girls, there were 7 black, 2 biracial (2 Asian-white), and 78 white. Seventy adolescent girls were referred for the evaluation of oligo/amenorrhea, acanthosis nigricans, or hirsutism. There were 5 black, 1 Asian, 1 biracial (black-white), and 63 white adolescent girls. For all subjects, congenital adrenal hyperplasia, Cushing's syndrome, and hyperprolactinemia were excluded by past medical history, physical examination, and laboratory data. Consecutive subjects, for whom parental consent was obtained, were recruited from 1994 to 2002. This protocol was approved by the Human Rights Committee of the Children's Hospital of Pittsburgh and the Institutional Review Board of the University of Pittsburgh. Parental consent and patient assent were obtained from all subjects.

Height, weight, pubertal stage, and clinical manifestations of hyperandrogenism were recorded for all subjects. Height was measured using a calibrated stadiometer. Weight was measured with the subject wearing light indoor clothing without shoes on calibrated scales. Body mass index (BMI) was calculated as weight (kg)/height (m)². BMI could not be calculated for the one girl with HA who was wheelchair bound due to limb girdle muscular dystrophy. BMI percentiles and Z scores were determined according to the charts for the United States published by the Centers for Disease Control and Prevention in 2000 (35).

All adolescent girls were documented to have hyperandrogenism which was defined as having Δ^4 -androstenedione, total testosterone, and/or free testosterone concentrations elevated for age and pubertal stage. Four pairs of sisters were studied. For three sibling pairs, the older sister presented in adolescence with hirsutism and the younger sister had PP. For one pair of sisters, both were referred for evaluation of PP. Prospective longitudinal data were available for 37 girls.

Methods

Blood samples obtained from the patients were assayed for Δ^4 -androstenedione, 17-hydroxyprogesterone, and cortisol as described previously (36). Testosterone, free testosterone, and sex hormone binding globulin (SHBG) were measured by Esoterix (Calabasas Hills, CA).

Blood samples for fasting glucose (GF) and insulin (IF) determinations were obtained in 75 girls (33 PP and 42 HA). The fasting glucose and insulin determinations were obtained during oral glucose tolerance tests for 54 girls (23 PP and 31 HA) as part of the study protocol. The homeostasis model assessment (HOMA) was used as a surrogate measure of insulin sensitivity.

HOMA-IR (homeostasis model assessment estimate of insulin resistance) was calculated as $(I_F \times G_F)/22.5$ using the computer based program (www.dtu.ox.ac.uk/homa) (37–39). Use of these indices was limited to the girls with normal glucose tolerance test results (fasting blood glucose < 100 mg/dl [5.6 mmol/L] and two hour glucose < 140 mg/dl [7.8 mmol/L]) (40). Fasting insulin and glucose determinations and oral glucose tolerance tests were added to the protocol as data accumulated regarding the importance of insulin resistance in the pathophysiology of PP and PCOS (4,5).

Blood samples used for extraction of genomic DNA were obtained from all the participants. Genomic DNA was extracted from peripheral blood lymphocytes. Molecular genotype analysis for the rs2236418 SNP in the *GAD2* gene was performed by gene specific PCR followed by allele specific RFLP analysis. Primers were: 5'-AGCTCCCTCCCTCTCTCGTGTTT-3' and 5'-TATGCGAGCTGGAGACAGGGTTTA-3' which yielded a 203 bp product. The specific alleles were differentiated by digestion with *DraI*. In the presence of the A allele, digestion yielded two fragments: 144 bp and 59 bp. This restriction site was lost with the variant G allele.

Statistical analysis

χ^2 contingency table analyses were used to compare allele frequencies between the groups. Independent and paired Student's t-tests and ANOVA were used to assess for potential differences in BMI, BMI Z-score, hormone concentrations, and insulin sensitivity between groups and between individuals with specific genotypes. Correlation matrices calculated Pearson's r. Statistical analysis was performed using AbSTAT statistical software (Anderson-Bell, Arvada, CO).

Results

Subjects

The mean chronologic age for the 87 girls with PP was 7.65 ± 1.36 years; the age range was 4.75 to 11.58 years. The mean chronologic age for the 70 girls who presented with clinical features of hyperandrogenism was 15.29 ± 1.94 years; the age range was 10.40 to 21.50 years.

Since BMI and hormone concentrations vary during the peri-pubertal years, the PP and HA subjects (Table 1) were classified into three groups according to chronological age at presentation. Group 1 consisted of 51 girls who were less than 8 years of age at initial presentation. All had been referred for evaluation of PP. Group 2 consisted of 40 girls between the ages of 8 and 12 years; 3 had been referred for hyperandrogenism and 37 had been referred for evaluation of PP. Group 3 consisted of 66 girls greater than 12 years of age; all had been referred for evaluation of hyperandrogenism.

Allele and Haplotype Frequencies

Including all the girls with PP, allele frequencies were 74.1% for the A allele and 25.9% for the G allele. Among the girls with PP, 53 (60.9%) girls were homozygous for the A allele, 23 (26.5%) girls were heterozygous, and 11 (12.6%) girls were homozygous for the G allele. With exclusion of the four younger sisters, allele frequencies were unchanged, 73.8% for the A allele and 26.2% for the G allele.

For the girls with HA, allele frequencies were 77.5% for the A allele and 22.5% for the G allele. Among the girls with HA, genotype analysis showed that 40 (58.0%) girls were homozygous for the A variant, 27 (39.1%) girls were heterozygous, and 2 (2.9%) girls were homozygous for the G variant. The girl with acanthosis nigricans was homozygous for the G variant. Excluding the four younger sisters, comparison of haplotype frequencies revealed that the

frequency of homozygosity for the G variant was significantly greater in the girls with PP compared to the HA group, $p<0.05$.

Among the 11 girls with PP and GG genotype, ethnic backgrounds were white ($n=6$) and black ($n=5$). The black girls with the GG genotype were significantly younger than the white girls, 7.0 ± 0.8 years vs 8.1 ± 0.8 years, $p<0.05$. BMI and BMI Z-score were significantly higher among the white girls compared to the black girls, 21.9 ± 3.9 kg/m² vs. 17.1 ± 1.1 kg/m², $p<0.05$, respectively, and 1.9 ± 1.0 vs. 0.6 ± 0.5 , $p<0.05$, respectively. Among the two girls with HA who were homozygous for the G variant, 1 girl was white and 1 girl was black.

When BMI values were expressed as Z-scores, no significant differences were found between the three age groups. As anticipated, androstenedione, 17-hydroxyprogesterone and testosterone concentrations and BMI increased with chronological age. HOMA-IR was significantly lower in the youngest girls in Group 1 (1.8 ± 1.0 ; $n=16$) compared to the other two older groups (Group 2, 3.8 ± 2.2 ; $n=18$ and Group 3, 4.2 ± 2.2 ; $n=40$), $p<0.005$.

Relationships between BMI, hormone concentrations, and genotype

Genotype frequencies for girls by age group are listed in Table 1. Within each age group, BMI, BMI Z-scores, and testosterone concentrations were comparable for all three genotypes (data not shown). Within each age group and with exclusion of the ten girls with impaired glucose tolerance (2 hour glucose values ≥ 140 mg/dl), fasting insulin concentrations, fasting glucose concentrations, and HOMA-IR were comparable among the genotypes.

Longitudinal Outcome

Thirty-seven (23.6%) of the 157 girls have been followed prospectively since their initial evaluations (Table 2). All have achieved menarche and are greater than 12 years of age. Thirty four (91.9%) girls had presented with premature pubarche and three (8.1%) girls had presented with hyperandrogenism. Two of the three girls who presented with hyperandrogenism reported that they had pubic hair development prior to age 8 years of age. Mean chronologic age at presentation was 8.2 ± 2.1 years. Mean chronologic age at most recent evaluation was 17.0 ± 2.9 years. Mean number of years of follow-up was 8.9 ± 2.9 years (median 8.7 years, minimum 2.7 year and maximum 15.1 years). Ethnic distribution was 1 black, 1 Asian-white, and 35 white. Of these 37 girls, 26 (70.3%) had persistent hyperandrogenism and 11 (29.7%) had normal androgen concentrations at their most recent visit. Mean number of years for follow-up was comparable between the girls with persistent hyperandrogenism and those with normal androgen concentrations (Table 2). Twenty two girls were homozygous for the A allele, ten were heterozygous, and five were homozygous for the G allele. Allele and genotype frequencies were comparable between the 26 girls with persistent hyperandrogenism and those with normal androgen concentrations. Mean number of years of follow-up was comparable among the three genotypes. Allele frequency in this subset of subjects did not differ from the 87 girls who presented with PP.

When the girls were classified by BMI Z-score at the initial visit, 27 girls had BMI Z-scores ≤ 2.0 and 10 girls had BMI Z-scores > 2.0 . At the most recent visit, BMI values were greater than 32 kg/m² for all girls with an initial BMI Z-score > 2.0 . BMI Z-score remained > 2.0 for nine of the 10 girls with initial BMI Z-score > 2.0 . For the tenth girl, her most recent BMI Z-score and BMI value were 1.81 and 33.29 kg/m², respectively. BMI values at initial visit correlated with BMI values at the most recent visit, Pearson's $r=0.82$, $p<0.0001$. BMI Z-score at initial visit also correlated with BMI Z-score at most recent visit, $r=0.89$, $p<0.0001$.

Androstenedione concentration at the initial visit correlated with BMI at the initial visit, $r=0.6085$, $p<0.0001$, and with BMI Z-score at the initial visit, $r=0.3741$, $p<0.05$. At the most

recent visit, there were no significant correlations between hormone concentrations and BMI or BMI Z-score.

Mean BMI at the initial visit was comparable among the three genotypes. At the most recent follow-up, mean BMI was significantly greater among the girls homozygous for GG compared to those homozygous for AA, $36.0 \pm 8.8 \text{ kg/m}^2$ vs. $24.7 \pm 5.7 \text{ kg/m}^2$, respectively, $p < 0.005$. At this most recent follow-up, mean BMI for girls with GA genotype was $29.5 \pm 7.6 \text{ kg/m}^2$, which was not statistically different from either the AA or GG genotype groups. Mean BMI for girls with either GG or GA genotype (G/*) was $31.7 \pm 8.3 \text{ kg/m}^2$ which was significantly greater than that for the girls homozygous for the A allele. There were no significant differences in BMI Z-scores between the three genotypes at either the first or most recent visit. Androstenedione and testosterone concentrations at the most recent visit were comparable among the three genotypes.

When girls were classified as either AA or G* (GG or AG) genotypes, chronologic age at presentation, most recent chronologic age, and duration of follow-up were comparable (Table 2). At the initial evaluation, mean BMI was significantly greater for girls with G* (GG or GA) genotype, $23.5 \pm 6.1 \text{ kg/m}^2$ vs. $18.7 \pm 3.4 \text{ kg/m}^2$, $p < 0.005$ (Figure 1). Mean BMI Z-score at the initial visit was also significantly greater in girls with G* genotype, 1.8 ± 1.4 vs. 0.9 ± 1.2 , $p < 0.05$. Mean BMI at most recent visit was significantly greater in girls with G* genotype, $31.7 \pm 8.3 \text{ kg/m}^2$ vs. $24.7 \pm 5.7 \text{ kg/m}^2$, $p < 0.005$. At the most recent visit, the BMI Z score among girls with G* genotype was higher than the girls with AA genotype, 1.5 ± 1.0 vs. 0.9 ± 0.9 , $p = 0.062$, but did not achieve statistical significance. Eleven of the 15 (73.3%) girls with G* genotype had a BMI $\geq 26 \text{ kg/m}^2$ at the most recent visit compared to 6/22 (27.3%) girls with AA genotype. Genotype (G* vs. AA) correlated with initial BMI and BMI at follow-up, $r = 0.455$, $p < 0.005$. When expressed as change in BMI over time in years, girls with GG genotype had a significantly greater increase in mean BMI over time compared to those with AA genotype, $1.4 \pm 0.8 \text{ kg/m}^2/\text{yrs}$ vs. $0.7 \pm 0.5 \text{ kg/m}^2/\text{yrs}$. For girls with GA genotype, mean BMI over time was $0.8 \pm 0.4 \text{ kg/m}^2/\text{yrs}$.

Of the 10 girls with BMI Z-scores > 2.0 at the initial visit, seven were homozygous or heterozygous for G. At the initial visit, frequencies of the G allele were 18.5% in the girls with BMI Z-scores ≤ 2.0 and 50% among the girls with BMI Z-scores > 2.0 . The frequency of the G allele was significantly greater in the girls with BMI Z-scores > 2.0 , $p < 0.05$.

Androstenedione, sex hormone binding globulin, basal 17-OHP, and ACTH-stimulated 17-OHP concentrations obtained at the initial visit were comparable between the AA and G* genotype groups. Androstenedione and testosterone concentrations were comparable at the follow-up visits for AA and G* genotype groups.

Fasting insulin and glucose determinations were available for 15 girls in the longitudinal group. Results of oral glucose tolerance tests were available for 12 girls. One girl had impaired glucose tolerance; she was homozygous for the G allele. Excluding this girl, fasting glucose values were significantly higher in girls with G* genotype, $86.1 \pm 7.1 \text{ mg/dl}$ vs. $77.0 \pm 3.6 \text{ mg/dl}$, $p = 0.0067$. Fasting insulin, HOMA-IR, 2 hour glucose, and 2 hour insulin concentrations were comparable between the two groups.

Discussion

It has become well established that genetic factors influence the propensity for obesity (41). Family studies of obesity have demonstrated linkage to a region on chromosome 10 where the *GAD2* gene is mapped (30,42–44). Boutin et al. reported that the (–243) A→G SNP located in the promoter region of the *GAD2* gene displayed a strong association with morbid obesity in their population of unrelated French subjects (32). However, other studies (Table 3)

involving Germans, British, American, and Canadian subjects showed no association between morbid obesity and this SNP (30,34,45). More recently, this SNP was assayed in 5857 middle-aged unrelated Danish White subjects; the G allele was modestly associated with lower BMI and lower fasting glucose concentrations (46). Inconsistent results have been obtained regarding the association of SNPs in the *GAD2* gene with body mass index and obesity in adults. In addition, investigations into the functional consequences of the (−243) A→G SNP in the promoter region of the *GAD2* gene have reported contradictory results (32,34).

In our population, the frequency of the GG genotype was higher in the girls with PP. Five of the seven (71%) black girls and six of 78 white (9.0%) girls were homozygous for the G allele. Among the girls with HA, only one black girl was homozygous for the G allele. However, available genotype information obtained from HapMap and Perlegen databases through NCBI (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=2236418) indicate that the G allele occurs more commonly in blacks than among whites. The higher frequency of the G allele in black girls with PP compared to those with HA could possibly be attributed to an underlying population/ethnic variation in the frequency of the G allele (47). An alternative explanation is that the higher frequency of the G allele represents ascertainment bias for referral of younger black girls for endocrine evaluation. The BMI Z-scores differences at presentation with greater BMI Z-scores among the white girls compared to the black girls may also reflect ascertainment bias.

In our study, we found no association of the (−243) A→G allele with androgen concentrations at any time-point. Allele frequencies reported for the G variant range from 16.0% in Canadian control subjects to 21% among obese French children and French adults (Table 3). In our longitudinal study, 20 (27%) of 74 alleles carried the G variant. When classified by BMI Z-score, the frequency of the G allele was 20.3% among girls with BMI Z-score ≤ 2.0 which is comparable to frequencies reported in other studies (Table 3). However, the frequency of the G allele was 50% in girls with initial BMI Z-score > 2.0. In our patient population, the presence of a G allele was associated with increased BMI Z-score at presentation and an increased propensity to maintain a higher BMI during the peripubertal years. Nevertheless, since only 10 girls presented with BMI Z-scores > 2.0, our findings may represent an ascertainment bias.

In both French children and adults, the GG genotype was associated with abnormal binge-eating behavior. Curiously, this variant was associated with lower birth weights in French infants with GG genotype. Low birth weight has been associated with increased risk for obesity in later childhood. Among these children, G* genotype was associated with increased BMI after age 11 years. They also reported a trend toward lower insulinogenic indices in children carrying a G allele. Similar to the findings in French children, we have found that the G allele is associated with increased BMI in late childhood and adolescence. Apart from the morbidly obese French adults, this association seems to disappear in most other adult populations studied to date. One potential explanation for the results in children is that different genes have greater influence in the development of obesity in children than in adults.

In summary, we found no association between androgen concentrations and the A→G SNP in the *GAD2* gene in our population of girls with PP and HA. Importantly, we found an association between the G allele and increased BMI in our population. In our longitudinal study with median follow-up over 8.5 years, the presence of one or more G alleles was associated with increased peripubertal weight gain. One unique aspect of our study is the longitudinal follow-up for the subjects. Additional prospective studies are essential to replicate our findings that in girls with PP, the presence of the G allele is associated with increased BMI in adolescent girls. If confirmed, girls with PP who carry the G allele can be targeted for lifestyle intervention.

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Abbreviations

PA	Premature adrenarche
PP	Premature pubarche
HA	hyperandrogenism
PCOS	polycystic ovary syndrome
SNP	single nucleotide polymorphism
BMI	body mass index
GF	fasting glucose
IF	fasting insulin
HOMA IR	homeostasis model assessment estimate of insulin resistance

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Figure 1.

BMI at both timepoints (initial evaluation and most recent visit) in longitudinally studied patients. The Y axis indicates BMI in kg/m^2 . BMI1 indicates BMI value at presentation. BMI2 indicates BMI value at most recent evaluation. The two timepoints for G^* patients are illustrated on the left side and the two timepoints for the AA patients are illustrated on the right side of the figure. Lines connect the BMI values for individual subjects.

Figure 2.

BMI Z-score at both timepoints in longitudinally studied patients. The Y axis indicates BMI Z-score. Z-BMI-1 indicates BMI Z-score value at presentation. Z-BMI-2 indicates BMI Z-score value at most recent evaluation. The two timepoints for G* patients are illustrated on the left side and the two timepoints for the AA patients are illustrated on the right side of the figure. Lines connect the BMI Z-score values for individual subjects.

Table 1

Descriptive features, hormone concentrations, allele and genotype frequencies for girls with PP and HA classified by age groups. Chronological age (CA), bone age (BA), and follow-up are expressed in years. Androstenedione (AND), testosterone, 17-hydroxyprogesterone (17-OHP), and ACTH-stimulated 17-OHP (17-OHP_30) concentrations are expressed as ng/dl. Sex hormone binding globulin (SHBG) concentrations are expressed in mcg/dl. Numbers in parentheses indicates number of subjects when less than the total group.

Group	1 (<8 yrs)	2 (8–2 yrs)	3 (>12 yrs)
CA	6.8±0.9 ^a	9.2±1.1 ^a	15.5±1.8 ^a
PP/HA	51/0	37/3	0/66
Race (W/B/Bi/A)	44/5/2/0	38/2/0/0	59/5/1/1
Breast Stage	1.1±0.4 ^a	2.0±1.3 ^a	4.8±0.6 ^a
Pubic Hair Stage	2.0±0.4 ^a	2.8±1.1 ^a	5.0±0.7 ^a
BMI (kg/m ²)	19.6±3.1 ^a	22.5±6.5 ^b	30.1±8.4 ^{a,b}
Z-BMI	1.6±1.2	1.4±1.7	1.8±1.6
AND-0	89.9±44.2 (50) ^{a,c}	146.7±99.7 (39) ^{b,c}	357.4±137.9 (62) ^{a,b}
17-OHP	82.8±62.8 (44) ^d	97.4±92.4 (34) ^e	144.1±93.5 (57) ^{d,e}
17-OHP_30	340±144 (37)	362.5±165.7 (29)	362.5±179.7 (56)
Testosterone	9.0±3.4 (19) ^b	20.1±36.6 (17) ^c	52.0±42.5 (32) ^{b,c}
SHBG	1.2±0.5 (26) ^f	1.0±0.7 (24)	0.7±0.7 (31) ^f
HOMA-IR	1.8±1.0 (16) ^g	3.7±2.1 (17) ^h	4.2±2.4 (30) ^{g,h}
Allele frequency			
A/G	77/25	55/25	104/28
Genotypes			
AA/AG/GG	33/11/7	20/15/5	40/24/2

^aKey: p<0.0001;

^bp=0.0001;

^cp=0.0001;

^dp=0.001;

^ep=0.001;

^fp<0.05;

^gp<0.005;

^hp<0.005; For race, W=white; B=black; Bi=biracial; A=Asian.

Table 2

Descriptive features, hormone concentrations, and genotypes for girls followed longitudinally. Chronological age (CA), bone age (BA), and follow-up are expressed in years. Androstenedione (AND), testosterone, and 17-hydroxyprogesterone (17-OHP) concentrations are expressed as ng/dl. Sex hormone binding globulin (SHBG) concentrations are expressed in mcg/dl. Data obtained at initial visit are indicated by “-1”. Data obtained at most recent visit are indicated by “-2”. P values for comparison of AA and G* (AG or GG) genotypes are listed in the column on the far right. Numbers in parentheses indicate number of subjects when less than the total group.

	All	AA	G*	P value
PP/HA	34/3	22	15	
CA-1	8.2±2.1	7.7±1.9	8.9±2.3	0.0860
CA-2	17.0±2.9	16.7±3.2	17.5±2.5	0.4233
Breast Stage	1.7±1.2	1.4±1.0	2.1±1.3	0.0697
Pubic Hair Stage	2.6±1.1	2.3±0.8	3.0±1.3	0.0254
Bone Age	9.4±1.6 (30)	9.2±1.3 (19)	9.8±2.0 (11)	0.2606
BMI-1 (kg/m ²)	20.6±5.2	18.7±3.4	23.5±6.1	0.0043
BMI-2 (kg/m ²)	27.5±7.6	24.7±5.7	31.7±8.3	0.0046
Z-BMI-1	1.3±1.3	0.9±1.2	1.8±1.4	0.0363
Z-BMI-2	1.1±1.0	0.9±0.9	1.5±1.0	0.0617
AND-1	127.6±84.8 (36)	115±59 (22)	148±114 (14)	0.2549
AND-2	258.5±123.6 (36)	258±113 (22)	260±143 (14)	0.9590
Testosterone-1	24.1±39.3 (15)	15±14 (10)	43±65 (5)	0.1893
Testosterone-2	32.0±13.8 (31)	32±14 (20)	32±15 (11)	0.9154
17-OHP-1	95.8±85.3 (34)	93±62 (19)	100±110 (15)	0.8195
SHBG	1.0±0.6 (25)	1.0±0.5 (17)	1.1±0.7 (8)	0.4963
HOMA-IR	2.8±1.8 (14)	2.0±0.5 (7)	3.1±1.9 (7)	0.1520
Follow-up (yrs)	8.9±2.9	9.0±2.5	8.6±3.5	0.6589

Table 3

Reported Allele Frequencies. The number of subjects studied is indicated in parentheses.

Population	A	G	Citation
Obese French Adults (867)	79%	21%	Boutin et al., 2003 (32)
French Adult Controls (646)	83%	17%	Boutin et al., 2003 (32)
Obese French Children (477)	79%	21%	Meyre et al., 2005 (33)
USA Cases (302)	81.6%	18.4%	Swarbrick et al., 2005 (34)
USA Controls (427)	83.1%	16.9%	Swarbrick et al., 2005 (34)
Canadian Cases (378)	84.0%	16.0%	Swarbrick et al., 2005 (34)
Canadian Controls (759)	82.5%	17.5%	Swarbrick et al., 2005 (34)
Obese Utah adults (855)	81.9%	18.1%	Hunt et al., 2006, (45)
Non-obese Utah adults (130)	80.8%	19.2%	Hunt et al., 2006, (45)
Middle-aged Danish adults (5857)	81.2%	18.8%	Boesgaard et al., 2007, (46)
BMI < 25 kg/m ² (2582)	80.5%	19.5%	
BMI > 30 kg/m ² (968)	82.9%	17.1%	
Longitudinal Group (37)			Witchel et al, this study
BMI Z-score ≤2.0 (27)	81.5%	18.5%	
BMI Z-score >2.0 (10)	50%	50%	