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Genetic variation of *FTO* and *TCF7L2* in premature adrenarche

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Abstract

Premature adrenarche (PA) has been associated with increased body mass index. Our aim was to determine whether the obesity-associated variant at fat mass and obesity gene (*FTO*) is more frequent in PA subjects. Furthermore, we hypothesized that altered Wnt signaling due to genetic variants at transcription factor 7–like 2 (*TCF7L2*) could play a role in the polygenic pathogenesis of PA. We genotyped polymorphisms at *FTO* rs9939609 and at *TCF7L2* rs7903146 and rs12255372 in 73 Finnish white prepubertal children with PA and in 97 age- and sex-matched healthy controls. In addition, we investigated the associations of these genetic variations with weight, height, circulating adrenocortical hormone levels, glucose metabolism, lipid profile, and blood pressure. The differences in the minor allele frequencies (MAFs) of rs9939609, rs7903146, and rs12255372 were not statistically significant between the PA and control groups (difference in MAFs [95% confidence interval]: −0.06 [−0.18, 0.05], 0.04 [−0.05, 0.12], and 0.01 [−0.07, 0.10]; P = .3, .4, and .8, respectively). However, the risk allele at *TCF7L2* rs7903146 was more frequent in PA subjects than in controls when we restricted the analysis to the subjects with lower weight-for-height than the median of the PA subjects (weight-for-height <108%, corresponding body mass index SD score <0.79; difference in MAFs [95% confidence interval]: 0.12 [−0.001, 0.23]; P = .038). Risk variant at *FTO* rs9939609 associated with higher weight-for-height in the healthy children (P = .001). In conclusion, the minor variant at *FTO* rs9939609 seems to play no major role in the increased weight-for-height of PA subjects; but the risk allele at *TCF7L2* rs7903146 may have a role in the pathogenesis of PA in lean subjects.

1. Introduction

Adrenarche means the gradual rise in adrenocortical production of androgens [1]. Pathogenesis of premature adrenarche (PA) is complex and polygenic, resulting in a wide spectrum of clinical signs including premature pubarche (PP) but also milder androgenic signs before the age of 8 years in girls and 9 years in boys [2,3]. Premature adrenarche has been connected with the risk of developing ovarian hyperandrogenism and features of metabolic syndrome [4-6]. Several studies have searched for genetic factors predisposing to PA in genes involved in steroid synthesis [7,8], androgen action [9,10], and insulin–insulin-like growth factor functions [11,12]. Although some associations have been found, the underlying susceptibility genes remain largely unknown.

Premature adrenarche associates with increased body mass index (BMI) in some populations [6,13], and it is well known that healthy obese children have elevated adrenal androgen levels compared with lean children [14]. However, the fat-related genetic variation in relation to the timing of adrenarche has been previously investigated only in studies on β_3-adrenergic receptor variant W64R [15] and glutamate decarboxylase 2 gene polymorphism −243A/G [16]. Genetic variants in the fat mass and obesity gene (*FTO*) have been associated consistently with obesity [17-19]. This association reflects an increase in fat mass that is observed from early infancy [20] to later childhood and upward [17,19].

The study protocol was approved by the Research Ethics Committee of Kuopio University Hospital.

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FTO genotype correlates also with traits of glucose and lipid metabolism to an extent consistent with its effect on BMI [21,22], leading to increased risk for metabolic syndrome [21] and type 2 diabetes mellitus [17].

In the search for other genetic factors associated with PA, we have turned our eyes to the WNT signaling, which is essential for embryogenesis, postnatal development, and tissue homeostasis (reviewed in He et al [23]). The Wnt signaling has been found to have an active role in the development and function of adrenal glands [24-26]. Wnt4 represses steroidogenesis in the adrenal cortex and gonads based on the studies on transgenic Wnt4 mice [27,28]. In humans, loss-of-function mutations in *WNT4* have been described in the autosomal recessive SERKAL syndrome with female to male sex reversal and renal, adrenal, and lung dysgenesis [29], and in women with müllerian-duct regression, virilization, and high androgen levels [30,31]. Schinner et al [32] found adipocyte-derived products to induce adrenal expression of the steroidogenic acute regulatory protein, and aldosterone and cortisol secretion through the Wnt signaling pathway; but adrenal androgens were not measured in that delicate study. In our previous study, we found an association between serum dehydroepiandrosterone sulfate (DHEAS) levels and low-density lipoprotein–related protein–5 (*LRP5*) polymorphisms A1330V and N740N in healthy prepubertal children, but observed no association between PA and genetic variation in *LRP5* that encodes a Wnt coreceptor [33]. Downstream in the canonical Wnt signaling pathway are the TCF transcription factors that activate WNT target gene expression with β-catenin; but in the absence of β-catenin, TCFs bind on the target gene promoters and repress their expression (reviewed in Jin [34]). One of the TCFs is transcription factor 7–like 2 (*TCF7L2*), the genetic variants of which have been previously associated with type 2 diabetes mellitus [35-37] and an increased risk for early impairment of glucose metabolism in obese children [38].

We hypothesized that the genetic variants in *FTO* and *TCF7L2* may be significant in the pathogenesis of PA. Therefore, we genotyped the *FTO* variant rs9939609 and *TCF7L2* variants rs7903146 and rs12255372 in 73 children with PA and in 97 age- and sex-matched controls. In addition, we examined the associations between the genetic variants and clinical-metabolic characteristics.

2. Subjects and methods

2.1. Subjects

The study group comprised 170 Finnish children. The recruitment of the subjects has been documented previously in more detail [39]. For the 73 subjects with PA (63 girls and 10 boys), the inclusion criteria were any clinical sign(s) of adrenarche, including pubic/axillary hair, acne, adult-type body odor, and oily hair, before the age of 8 years in girls and 9 years in boys. Steroidogenic enzyme defects and virilizing tumors were excluded biochemically and by adrenal ultrasonography. Altogether 97 (79 girls and 18 boys) age- and sex-matched healthy controls, representing a random sample of children from the same hospital district, were identified through the Finnish population register. At examination, girls in both groups had to be younger than 9 years and boys younger than 10 years. Children with central puberty, any endocrine disorder, or long-term medication were excluded from both groups. The study protocol was approved by the Research Ethics Committee of Kuopio University Hospital. Informed written consent from parents and assent from children were obtained for participation in the study, including collection and genotyping of DNA samples.

2.2. Clinical and endocrine-metabolic assessment

The time of appearance of the adrenarchal signs was obtained by interviewing the parents. Birth weight, birth length, and gestational age data were obtained from hospital records. The birth measures were converted to SD scores (SDS) by plotting them on the growth charts and adjusting the birth measures for sex and duration of gestation [40]. Weight was converted to percentages in relation to the median weight-for-height according to the national reference values [41]. Blood pressure (BP) was measured with a standard sphygmomanometer from the left arm in supine position after a 30-minute rest in bed and recorded as the average of 3 repeated measurements.

An intravenous cannula was placed for sampling. Baseline levels of plasma glucose, total, low-density (LDL) and high-density lipoprotein (HDL) cholesterol, triglycerides, serum insulin, cortisol, DHEAS, dehydroepiandrosterone, androstenedione, and sex hormone–binding globulin (SHBG) were measured between 9:00 and 10:00 AM for all subjects after an overnight fast. The assays for hormone measurements have been reported previously [6,39]. An oral glucose tolerance test was performed by administering 1.75 g/kg glucose (maximum of 75 g) to each subject, with samples for glucose and insulin analyses taken at 30, 60, 90 and 120 minutes and analyzed as previously reported [6].

2.3. Genotyping

DNA samples were available for all the subjects. DNA was isolated from peripheral blood samples using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI). Genotyping of rs9939609 of *FTO* (located in intron 1) and of rs12255372 (intron 4) and rs7903146 (intron 3) of *TCF7L2* was carried out using TaqMan Allelic Discrimination Assays (Applied Biosystems, Foster City, CA). Primers are available as Supplementary Material Online. TaqMan genotyping reaction was amplified on a GeneAmp PCR system 2700, and fluorescence was detected using an ABI Prism 7000 sequence detector (Applied Biosystems). Genotyping success rate was 100%.

Table 1
Cohort characteristics and MAFs of genetic variants at *FTO* and *TCF7L2*

	Controls	PA subjects		*P*
n	97	73		
Sex (boys/girls)	18/79	10/63		.40[a]
Age (y)	7.5 ± 0.9	7.5 ± 0.9		.77[b]
Weight-for-height (%)	107 ± 15	114 ± 19		.004[c]
Height SDS	0.3 ± 1.0	1.2 ± 1.1		<.001[b]
MAFs (%)			Difference in proportions (95% CI)[d]	*P*[e]
FTO				
rs9939609	42.8	36.3	−0.06 (−0.18, 0.05)	.27
TCF7L2				
rs7903146	13.9	17.8	0.04 (−0.05, 0.12)	.41
rs12255372	14.9	16.4	0.01 (−0.07, 0.10)	.82

[a] *P* value was taken from χ^2 test.
[b] Values are reported as mean ± SD; *P* values were taken from Student *t* test.
[c] Values are reported as mean ± SD; *P* value was taken from Mann-Whitney test.
[d] 95% CI for the differences in the minor allele proportions.
[e] *P* values were taken from 2-sample test for equality of proportions with continuity correction.

2.4. Statistical analyses and power calculations

The significance in the differences of the minor allele frequencies (MAFs) between the groups was tested with the 2-sample test for equality of proportions with continuity correction giving the 95% confidence intervals (CIs) for the differences. The difference in sex distribution between the PA and control groups was tested with χ^2 test. The relationships between *FTO* genotype group and phenotype were estimated using multiple linear regression allowing for age and sex. Because there were no more than 2 homozygotes for the minor allele at *TCF7L2* rs7903146 in the PA or control groups, the heterozygotes and homozygotes were combined in the statistical analysis. Associations between the *TCF7L2* polymorphism and clinical and biochemical parameters were examined in the PA and control groups using univariate linear model incorporating age and sex as covariates. If the distribution of the variable being tested was not normal, raw data were log-transformed before using the multiple linear regression or univariate linear model; and the group characteristics are presented as geometric means with 95% CI. For age, birth measures, and height SDS, analysis of variance and Student *t* test were used to test differences between genotype groups and case-control groups; for weight-for-height, the Kruskall-Wallis and Mann-Whitney nonparametric tests were used. To compare lean PA subjects with those with higher weight-for-height, we separated the groups by the median weight-for-height of the PA subjects (108%) to get equal-sized groups. *P* less than .05 was considered statistically significant. Statistical analyses were performed with SPSS 14.0 statistical package (SPSS, Chicago, IL), except for 2-sample test for equality of proportions and power calculations that were performed with R statistical program,

Table 2
Clinical characteristics according to the genotypes of *FTO* variant rs9939609 in control and PA children

Characteristic, mean (95% CI)	Controls				PA subjects			
	T/T	T/A	A/A	*P*[a]	T/T	T/A	A/A	*P*[a]
No. of subjects (%)	31 (32)	49 (50)	17 (18)		30 (41)	33 (45)	10 (14)	
Age (y)[b]	7.5 (7.1, 7.8)	7.5 (7.2, 7.7)	7.6 (7.1, 8.0)	.95	7.3 (7.1, 7.6)	7.5 (7.2, 7.9)	7.5 (6.6, 8.4)	.70
Birth weight SDS[b]	0.03 (−0.3, 0.4)	0.2 (−0.1, 0.5)	0.4 (−0.2, 1.0)	.52	−0.1 (−0.5, 0.3)	−0.1 (−0.4, 0.3)	0.4 (−0.8, 1.6)	.45
Birth length SDS[b]	0.3 (−0.1, 0.6)	0.2 (−0.06, 0.5)	0.4 (−0.03, 0.9)	.77	−0.1 (−0.5, 0.3)	−0.07 (−0.4, 0.3)	0.3 (−0.8, 1.3)	.62
Weight-for-height (%)[c]	99 (95, 103)	109 (105, 113)	112 (103, 121)	*.001*	112 (106, 119)	113 (106, 120)	125 (112, 137)	.18
Height SDS[b]	0.1 (−0.2, 0.5)	0.5 (0.2, 0.7)	0.2 (−0.3, 0.7)	.31	1.1 (0.6, 1.5)	1.2 (0.8, 1.5)	1.4 (0.6, 2.2)	.72
DHEAS (μmol/L)[d]	1.0 (0.6, 1.0)	0.9 (0.7, 1.0)	0.8 (0.6, 1.1)	.73	1.6 (1.3, 2.0)	1.7 (1.4, 2.1)	2.3 (1.4, 3.8)	.22
Δ4-A (nmol/L)[d]	1.2 (0.9, 1.4)	1.4 (1.2, 1.7)	1.4 (1.0, 1.8)	.26	2.5 (2.1, 3.0)	2.3 (1.9, 2.8)	2.7 (1.7, 4.2)	.73
Cortisol (nmol/L)[d]	226 (199, 257)	225 (201, 252)	237 (199, 283)	.73	248 (211, 290)	240 (205, 281)	173 (109, 274)	.06
SHBG (nmol/L)	107 (97, 116)	101 (92, 109)	95 (76, 115)	.22	87 (72, 101)	80 (67, 94)	70 (49, 92)	.38
Cholesterol (mmol/L)	4.3 (4.0, 4.6)	4.2 (4.0, 4.4)	4.2 (3.9, 4.5)	.75	4.2 (4.0, 4.5)	4.3 (4.1, 4.6)	4.1 (3.6, 4.5)	.90
LDL (mmol/L)	2.4 (2.2, 2.6)	2.5 (2.3, 2.7)	2.5 (2.2, 2.8)	.48	2.5 (2.3, 2.7)	2.6 (2.4, 2.8)	2.5 (2.2, 2.8)	.91
HDL (mmol/L)	1.6 (1.5, 1.7)	1.4 (1.3, 1.5)	1.5 (1.3, 1.6)	.09	1.3 (1.2, 1.5)	1.5 (1.3, 1.6)	1.3 (1.1, 1.6)	.66
Triglycerides (mmol/L)[d]	0.52 (0.45, 0.59)	0.58 (0.53, 0.65)	0.61 (0.49, 0.77)	.10	0.64 (0.54, 0.69)	0.61 (0.54, 0.69)	0.65 (0.54, 0.77)	.83
HOMA-IR[d]	0.8 (0.7, 0.9)	0.9 (0.8, 1.1)	0.9 (0.7, 1.1)	.39	1.0 (0.9, 1.2)	1.1 (0.9, 1.3)	1.4 (1.0, 1.8)	.23
ISI_{comp}	1.2 (1.0, 1.3)	1.1 (1.0, 1.2)	1.0 (0.8, 1.3)	.20	0.9 (0.7, 1.1)	0.8 (0.7, 1.0)	0.8 (0.6, 1.0)	.57
Systolic BP (mm Hg)	98 (96, 101)	102 (99, 105)	100 (95, 105)	.49	104 (101, 107)	104 (100, 108)	109 (101, 116)	.27

Homeostasis model assessment for insulin resistance was calculated according to the following formula: fasting plasma glucose (in millimoles per liter) ∗ fasting serum insulin (in microunits per milliliter)/22.5. Insulin sensitivity index = 10 000/√ [fasting glucose (in milligrams per deciliter) ∗ fasting insulin (in microunits per liter) ∗ mean glucose (in milligrams per deciliter) ∗ mean insulin (in microunits per liter)]. Δ4-A indicates androstenedione.
[a] Values were taken from multiple linear regression, age and sex as covariates, except for variables marked with [b] and [c].
[b] Analyzed with 1-way analysis of variance.
[c] Analyzed with Kruskall-Wallis nonparametric test.
[d] Raw data were log-transformed before using the univariate linear model, and results are presented as geometric means with 95% CI.

Table 3
Clinical characteristics according to the genotypes of *TCF7L2* variant rs7903146 in control and PA children

Characteristic, mean (95% CI)	Controls			PA subjects		
	C/C	C/T & T/T	P^a	C/C	C/T & T/T	P^a
No. of subjects (%)	71 (73)	26 (27)		49 (67)	24 (33)	
Age (y)[b]	7.5 (7.3, 7.7)	7.5 (7.2, 7.7)	.66	7.5 (7.2, 7.8)	7.4 (7.0, 7.8)	.54
Birth weight SDS[b]	0.2 (−0.3, 0.5)	0.04 (−0.4, 0.4)	.45	−0.04 (−0.4, 0.3)	−0.02 (−0.5, 0.4)	.95
Birth length SDS[b]	0.3 (0.1, 0.6)	0.1 (−0.3, 0.5)	.27	0.04 (−0.4, 0.3)	−0.05 (−0.5, 0.4)	.97
Weight-for-height (%)[c]	105 (102, 108)	111 (103, 118)	.20	116 (111, 121)	111 (102, 119)	.16
Height SDS[b]	0.4 (0.1, 0.6)	0.1 (−0.1, 0.3)	.14	1.1 (0.8, 1.5)	1.2 (0.8, 1.7)	.69
DHEAS (μmol/L)[d]	0.8 (0.7, 1.0)	0.7 (0.6, 0.9)	.47	1.7 (1.4, 2.0)	1.8 (1.4, 2.4)	.47
Δ4-A (nmol/L)[d]	1.4 (1.2, 1.6)	1.2 (1.0, 1.6)	.57	2.5 (2.1, 2.9)	2.3 (1.8, 2.9)	.67
Cortisol (nmol/L)[d]	237 (218, 258)	204 (175, 237)	.08	225 (196, 259)	248 (205, 300)	.34
SHBG (nmol/L)	101 (94, 109)	102 (90, 114)	.93	82 (70, 93)	82 (68, 96)	.99
Cholesterol (mmol/L)	4.2 (4.0, 4.4)	4.3 (4.0, 4.6)	.64	4.3 (4.1, 4.5)	4.2 (4.0, 4.5)	.79
LDL (mmol/L)	2.4 (2.3, 2.6)	2.6 (2.4, 2.9)	.18	2.6 (2.4, 2.8)	2.5 (2.3, 2.7)	.47
HDL (mmol/L)	1.5 (1.4, 1.6)	1.4 (1.3, 1.5)	.14	1.4 (1.3, 1.5)	1.4 (1.3, 1.5)	.56
Triglycerides (mmol/L)[d]	0.56 (0.51, 0.62)	0.56 (0.50, 0.63)	.84	0.66 (0.60, 0.74)	0.55 (0.48, 0.65)	*.03*
HOMA-IR[d]	0.9 (0.8, 1.0)	0.9 (0.8, 1.0)	.87	1.1 (0.9, 1.3)	1.2 (1.0, 1.4)	.50
ISI_{comp}	1.1 (1.0, 1.2)	1.1 (0.9, 1.2)	.77	0.9 (0.7, 1.0)	0.8 (0.7, 0.9)	.38
Systolic BP (mm Hg)	100 (97, 102)	102 (99, 106)	.08	105 (102, 107)	104 (99, 109)	.91
Subjects with weight-for-height <108%						
No. of subjects (%)	53 (82)	12 (18)		23 (62)	14 (38)	
Age (y)[b]	7.6 (7.4, 7.8)	7.5 (7.1, 8.0)	.8	7.4 (6.9, 7.8)	7.2 (6.7, 7.8)	.7
DHEAS (μmol/L)[d]	0.8 (0.7, 1.0)	0.7 (0.5, 0.9)	.3	1.4 (1.1, 1.8)	1.7 (1.2, 2.6)	.2
Triglycerides (mmol/L)[d]	0.55 (0.50, 0.61)	0.51 (0.43, 0.62)	.5	0.63 (0.53, 0.75)	0.53 (0.44, 0.63)	.1
HOMA-IR[d]	0.8 (0.7, 0.9)	0.8 (0.7, 0.9)	.8	0.8 (0.7, 1.0)	1.1 (0.9, 1.3)	.08
ISI_{comp}	1.2 (1.1, 1.3)	1.2 (1.1, 1.3)	.9	1.1 (0.9, 1.3)	0.8 (0.7, 1.0)	.1

Homeostasis model assessment for insulin resistance was calculated according to the following formula: fasting plasma glucose (in millimoles per liter) ∗ fasting serum insulin (in microunits per milliliter)/22.5. Insulin sensitivity index = 10 000/√ [fasting glucose (in milligrams per deciliter) ∗ fasting insulin (in microunits per liter) ∗ mean glucose (in milligrams per deciliter) ∗ mean insulin (in microunits per liter)].

[a] Values were taken from univariate linear model, age and sex as covariates, except for variables marked with [b] and [c].

[b] Analyzed with Student *t* test.

[c] Analyzed with Mann-Whitney *U* test.

[d] Raw data were log-transformed before using the univariate linear model, and results are presented as geometric means with 95% CI.

version 2.7.2 (http://www.r-project.org/). Hardy-Weinberg equilibrium was calculated according to standard procedures using χ^2 test. Linkage disequilibrium between *TCF7L2* polymorphisms was analyzed with Haploview 4.0 [42].

Sample size provided only 14% and 11% power to detect the observed differences in the proportions of minor alleles at rs9939609 and rs7903146, respectively ($\alpha = .05$). To prove the observed differences in the proportions of minor alleles at rs9939609 and rs7903146 with 80% power, we would have needed 892 and 1383 subjects in each group, respectively ($\alpha = .05$).

3. Results

We genotyped the *FTO* variant rs9939609 and the *TCF7L2* variants rs7903146 and rs12255372 in the total of 170 children: 73 with PA (63 girls and 10 boys) and 97 healthy prepubertal controls (79 girls and 18 boys). Clinical and laboratory characteristics of the study groups and the MAFs are given in Table 1. As reported in our previous studies [6,39], there was no significant difference in the sex distribution or age between the PA and control groups, whereas the PA subjects had higher weight-for-height and height SDS than the controls did (Table 1).

The MAFs of rs9939609, rs7903146, and rs12255372 were similar between the PA and control groups (Table 1). All the single nucleotide polymorphisms followed the Hardy-Weinberg equilibrium with a *P* value greater than .05 in both the PA subjects and controls. Linkage disequilibrium between the genotypes at *TCF7L2* rs7903146 and rs12255372 was high ($r^2 = 0.82$, D' 0.90). Therefore, in further statistical analyses, we focused on rs7903146, which has the strongest association with type 2 diabetes mellitus [43].

The minor variant at *FTO* rs9939609 associated significantly with higher weight-for-height in the control children (Table 2). A similar although nonsignificant trend was observed in the PA subjects. The minor variant at rs9939609 did not associate with birth measures, height SDS, adrenocortical hormone levels, BP, lipid profile, or measures of glucose metabolism in either control or PA group (Table 2).

Birth measures, current weight-for-height, height SDS, measures of adrenocortical function, homeostasis model assessment for insulin resistance (HOMA-IR), and insulin

sensitivity index (ISI_{comp}) were similar between the *TCF7L2* rs7903146 genotype groups in the control and PA subjects (Table 3). The PA subjects with the minor variant at rs7903146 had lower triglyceride level than the PA subjects with the major variant. When we used multiple linear regression model to test the associations of age, sex, weight-for-height, and *TCF7L2* genotype with the triglyceride level, the effects of sex and *TCF7L2* remained statistically significant (P = .029 and .046, respectively), whereas the effects of age and weight-for-height were statistically nonsignificant. The risk allele of rs7903146 was more frequent in PA subjects than in controls when we restricted the analysis on the subjects with lower weight-for-height than the median of the PA subjects (weight-for-height <108%, corresponding BMI SDS <0.79; controls, n = 65 vs PA, n = 37; difference in MAFs [95% CI]: 0.12 [−0.001, 0.23]; P = .038). When comparing parameters of glucose metabolism in these lean PA subjects, there were trends for higher HOMA-IR and lower ISI_{comp} in subjects with the risk allele at rs7903146 (Table 3).

When the groups formed by the DHEAS cutoff level of 1 μmol/L were analyzed, the MAFs of *FTO* rs9939609 and *TCF7L2* rs7903146 were similar in these 2 groups (DHEAS <1 μmol/L, n = 70 vs DHEAS ≥1 μmol/L, n = 100; difference in MAFs [95% CI]: 0.02 [−0.09, 0.14] and −0.04 [−.12, 0.05]; P = .7 and .4, respectively). The only statistically significant clinical or biochemical difference was in weight-for-height between the *FTO* genotype groups in the children with DHEAS less than 1 μmol/L (T/T n = 27, T/A n = 32 vs A/A n = 11; weight-for-height [percentage]: 98 [94, 102], 109 [104, 114] vs 109 [99, 119]; P = .001). No other significant differences in any of the studied parameters were observed between the *FTO* and *TCF7L2* genotype groups either in children meeting the biochemical criterion of adrenarche or in those with low DHEAS.

4. Discussion

We hypothesized that the genetic variation at *FTO* and *TCF7L2* may be significant in the pathogenesis of PA. Genetic variants at *FTO* rs9939609 and at *TCF7L2* rs7903146 and rs12255372 were not found to predispose to PA. However, the minor variant at *TCF7L2* rs7903146 was found to be more frequent in lean PA subjects in comparison with controls with the same weight-for-height. Risk variant at *FTO* rs9939609 associated with higher weight-for-height in the healthy prepubertal children. Neither *FTO* variant nor *TCF7L2* variants associated with clinical-metabolic parameters in our prepubertal PA subjects.

Risk variant at *FTO* rs9939609 was found to play no major role in the interplay between weight and PA. The significantly higher weight-for-height associating with the minor variant of *FTO* rs9939609 in the control group was in agreement with the previous large genome-wide association studies [17,19]. Although our prepubertal PA girls had higher mean weight-for-height than their controls [6], Spanish PP girls have been demonstrated to have increased central fat mass even with normal BMI throughout puberty [44]. Increased central fat mass has been suggested to relate to androgenemia and insulinemia, on which metformin treatment has been demonstrated to have beneficial effects in a follow-up study on Spanish girls with exaggerated adrenarche and low birth weight [45]. The previous study of Witchel and coworkers [16] associated the risk variant of polymorphism −234A/G at the glutamate decarboxylase 2 gene with increased BMI in girls with PP at both initial and follow-up visits, but the differences in the allele distribution were not tested in comparison with healthy subjects. The mechanisms by which increased weight-for-height and central fat mass would activate adrenal androgen secretion are incompletely understood, although hyperinsulinism is likely to contribute. In addition, the effects of adrenal androgens on adipose tissue and fat accumulation in PA have not been excluded. Besides being a target of hormonal actions, adipose tissue is known to be a major site for metabolism of sex steroids, among others, adrenal androgens [46]. In polycystic ovary syndrome patients, a vicious cycle has been suggested to exist whereby androgen excess favoring the abdominal deposition of fat further facilitates androgen secretion by ovaries and adrenals [47].

Mechanisms leading to clinical androgen signs and increased adrenal androgen levels might differ in lean PA subjects from those in PA subjects with higher BMI. In our previous study, we demonstrated that lean PA subjects have more active androgen receptors than those with higher BMI [10]. Based on the same division by median BMI in the PA group with whole clinical spectrum, the minor variant of *TCF7L2* rs7903146 was found to be more frequent in lean PA subjects in comparison with controls with the same weight-for-height. The biochemical mechanisms by which this single nucleotide polymorphism in the intron region might influence the expression of *TCF7L2* or the function of WNT signaling remain wholly unknown. The genetic variants in *TCF7L2* have been associated with decreased insulin secretion, which is linked to impaired incretin effects and β-cell proliferation [34]. A recent genome-wide association study on nearly 2000 diabetic patients and 3000 controls found the risk variants at *TCF7L2* to associate with an increased risk for type 2 diabetes mellitus in the subjects with lower BMI than the median BMI of the cases, whereas the risk ratio was lower in the subjects with higher BMI [48]. In our lean PA subjects, there were weak trends for higher HOMA-IR and lower ISI_{comp} in subjects with the risk allele in rs7903146, suggesting a possible role for genetic variation in *TCF7L2* in glucose metabolism. In nondiabetic children, the effects of risk variant at rs7903146 on glucose metabolism have been previously studied only in obese children. Körner et al [38] reported that the risk allele of rs7903146 was associated with higher fasting and 120-minute blood glucose in an oral glucose tolerance test in a cohort of 283 obese children with a mean age of 11.9

years and mean BMI SDS of 2.8. The statistically significant association between the minor variant at rs7903146 and lower triglyceride level is in contrast to recent findings on families with familial combined hyperlipidemia showing the minor variant to associate with higher triglyceride levels [49]. The discrepancy may reflect differences in the study groups, or our result may represent a false-positive finding due to small sample size.

Although our PA study group is among the biggest ones reported thus far, power calculations indicate that group sizes would need to be 10 times bigger to prove the negative results. Therefore, we are not able to exclude possible associations between PA and minor variants at *FTO* rs9939609 and *TCF7L2* rs7903146. The confidence intervals for the differences in the minor allele proportions would suggest that the risk variant at *FTO* is less frequent in PA subjects and the risk variant at *TCF7L2* rs7903146 is more frequent in comparison with healthy children. The value of this study lies on the precise phenotyping of both PA subjects and healthy controls. The population of Eastern Finland is known to be very homogenous genetically, which may be beneficial in the studies on complex traits [50,51].

In summary, the obesity-associated genetic variant at *FTO* rs9939609 was not associated with PA. Taking the limited power of this study into account, we cannot exclude the possible role of risk variant at *TCF7L2* rs7903146 in the pathogenesis of PA in lean subjects.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2009.03.025.

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