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Polymorphisms of *POR*, *SULT2A1* and *HSD11B1* in children with premature adrenarche

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ABSTRACT

Premature adrenarche (PA) refers to an earlier than normal increase in adrenocortical androgen production. The pathogenesis of PA remains largely unknown. We hypothesized that common polymorphisms at P450 oxidoreductase (*POR*), steroid sulfotransferase (*SULT2A1*), or 11β-hydroxysteroid dehydrogenase type 1 (*HSD11B1*) genes could contribute to the polygenic pathogenesis of PA. We performed a case–control study on the polymorphisms rs1057868 at *POR*, rs182420 at *SULT2A1*, and rs12086634 at *HSD11B1*. The study cohort comprised 73 prepubertal children with PA (defined by clinical signs) and 97 age- and gender-matched healthy controls from a Finnish Caucasian population. Genotype distributions and clinical and metabolic phenotypes were determined. The genotype distributions of the polymorphisms were similar between the study groups. No variant was associated with alterations in serum adrenal steroid concentrations. The minor C variant at *SULT2A1* was associated with higher serum sex hormone binding globulin (SHBG) concentrations (T/T, $n=64$ vs T/C&C/C, $n=33$; mean 94 vs 116 nmol/L; $P=.001$) and a trend for lower dehydroepiandrosterone sulfate/dehydroepiandrosterone ratios in the controls ($P=.06$), and with higher plasma total cholesterol concentrations in the PA subjects (T/T, $n=42$ vs T/C&C/C, $n=31$; 4.0 vs 4.6 mmol/L; $P<.001$). The minor G variant at *HSD11B1* was associated with lower plasma triglyceride concentration in the controls (T/T, $n=65$ vs T/G&G/G, $n=32$; 0.61 vs 0.49 mmol/L; $P=.013$). Common polymorphisms at *POR*, *SULT2A1* or *HSD11B1* were not associated with PA in a Finnish Caucasian population.

1. Introduction

In premature adrenarche (PA), signs of androgen action appear before the age of 8 years in girls or 9 years in boys [1,2]. The cause of PA is unclear and apparently polygenic.

In the adrenal cortex, 17α-hydroxylation and 17,20-lyase reactions are needed for androgen synthesis. For catalysis, CYP17A1 requires P450 oxidoreductase (POR) as an electron donor. The common single nucleotide polymorphism (SNP) rs1057868 at *POR* decreases CYP17A1 activity [3]. The last step

Abbreviations: CYP17A1, 17α-hydroxylase/17,20-lyase; DHEA(S), dehydroepiandrosterone (sulfate); HSD11B1, 11β-hydroxysteroid dehydrogenase type 1; HOMA-IR, homeostasis model assessment for insulin resistance; ISI_{comp}, insulin sensitivity index; MBS, metabolic syndrome; PA, premature adrenarche; PCOS, polycystic ovary syndrome; POR, P450 oxidoreductase; SHBG, sex hormone binding globulin; SNP, single nucleotide polymorphism; SULT2A1, steroid sulfotransferase.

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in the synthesis of dehydroepiandrosterone sulfate (DHEAS), the most adrenal-specific androgen, is catalyzed by the steroid sulfotransferase SULT2A1. The common rs182420 polymorphism at *SULT2A1* leads to decreased sulfotransferase activity, as shown in women with polycystic ovary syndrome (PCOS) [4]. Biologically inactive cortisone is converted to active cortisol by the 11β-hydroxysteroid dehydrogenase type 1 enzyme (HSD11B1). In lean PCOS subjects, the minor G variant of rs12086634 at *HSD11B1* associates with decreased enzyme activity and contributes to enhanced cortisol clearance and compensatory adrenal hyperandrogenism [5]. Together with another polymorphism, this variant may also protect against metabolic syndrome (MBS) [6].

We hypothesized that polymorphisms at *POR*, *SULT2A1*, and *HSD11B1*, all potential modulators of adrenal androgen production, could play a role in PA, which has been linked to an increased risk of ovarian hyperandrogenism and unfavorable metabolic features [7–9].

2. Subjects and methods

2.1. Subjects

The study group comprised 170 Finnish Caucasian children. The recruitment and assessment of the subjects have been documented previously [7,10]. For the 73 PA subjects (63 girls), the inclusion criteria were any clinical sign(s) of adrenarche (pubic/axillary hair, acne, adult type body odor, oily hair) before 8 years of age in girls and 9 years of age in boys. Steroidogenic enzyme defects and virilizing tumors were excluded biochemically and by adrenal ultrasonography. Altogether 97 (79 girls) age- and sex-matched healthy controls were identified through the Finnish population register. At examination, girls had to be <9and boys <10 years of age. Children with central puberty, any endocrine disorder, or long-term medication were excluded. The study protocol was approved by the Research Ethics Committee of Kuopio University Hospital. An informed written consent from the parents and an assent from the child were obtained before participation in the study.

2.2. Assessments

The time of appearance of adrenarcheal signs was obtained by interviewing the parents and by clinical examination [7,10]. Weight was converted to percentages in relation to the median weight-for-height by the national reference [11]. Plasma glucose and lipids, serum insulin, cortisol, DHEAS, DHEA, androstenedione and SHBG concentrations were measured between 0900 and 1000 h, after an overnight fast. The assays for serum hormone concentrations and the performance of the oral glucose tolerance test have been reported previously [7,10,12]. Insulin sensitivity index (ISI_{comp}) was calculated according to the formula: 10,000/√[fasting glucose(mg/dL) × fasting insulin(μU/mL) × mean glucose(mg/dL) × mean insulin(μU/mL)], and homeostasis model assessment for insulin resistance (HOMA-IR) according to the formula: fasting plasma glucose(mmol/l) × fasting serum insulin(μU/mL)/22.5.

DNA was isolated from peripheral blood samples using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI). Genotyping of rs1057868 at *POR* , rs182420 at *SULT2A1*, and rs12086634 at *HSD11B1* was carried out using TaqMan Allelic Discrimination Assays (Applied Biosystems, Foster City, CA). Primers are available on request. TaqMan genotyping reaction was amplified on a GeneAmp PCR system 2700 and fluorescence was detected using an ABI Prism 7000 sequence detector (Applied Biosystems). The genotyping success rate was 100%.

2.3. Statistical analyses

The difference in genotype distribution between the study groups was tested with the χ^2 test. Depending on the distribution of the variables and number of groups, the *t* test, ANOVA, Mann–Whitney or Kruskal–Wallis test was used to test differences of continuous variables between the study groups or the genotype subgroups (age and weight). Other associations between genotype and clinical parameters were examined using ANCOVA with age and sex as covariates. If the distribution of residuals of the variable being tested was not normal, raw data were log-transformed before the analysis, and the variable presented as

Table 1 – Group characteristics and genotype distributions of polymorphisms at *POR*, *SULT2A1*, and *HSD11B1* in children with premature adrenarche (PA) and in age- and gender matched controls.

		Median (range)	Controls (*n*=97)	PA subjects (*n*=73)	*P*
Characteristic		Sex (boys/girls)	18/79	10/63	.40*
		Age at examination (y)	7.6 (5.1, 8.9)	7.5 (4.8, 9.9)	.77**
		Weight-for-height (%)	102 (81, 159)	108 (89, 178)	.004†
		DHEAS (μmol/L)	0.7 (0.1, 4.4)	1.7 (0.4, 7.7)	<.001#
		DHEAS/DHEA ratio	200 (30, 1000)	250 (90, 580)	.004#
		Cortisol (nmol/L)	220 (92, 684)	231 (45, 680)	.75#
		Cholesterol (mmol/L)	4.2 (2.8, 6.8)	4.2 (2.7, 5.8)	.89**
Gene	Polymorphism				
POR	rs1057868	Genotype distribution (C/C, C/T, T/T; %)	40, 43, 17	37, 48, 15	.87*
SULT2A1	rs182420	Genotype distribution (T/T, T/C, C/C; %)	66, 30, 4	57, 32, 11	.20*
HSD11B1	rs12086634	Genotype distribution (T/T, T/G, G/G; %)	67, 31, 2	59, 33, 7	.23*

P values taken from *χ^2 test, **t test, †Mann–Whitney non-parametric test, #t test after log-transformation of the variable.
DHEAS, dehydroepiandrosterone sulfate; DHEA, dehydroepiandrosterone; *POR*, P450 oxidoreductase; *SULT2A1*, steroid sulfotransferase 2A1; *HSD11B1*, 11β-hydroxysteroid dehydrogenase type 1.

geometric mean ± 95% CI. $P < .05$ was considered statistically significant. Statistical analyses were performed with SPSS 17.0 statistical package for Windows or 16.0 statistical package for Mac (SPSS, Chicago, IL). Hardy–Weinberg equilibrium was calculated according to standard procedures using the χ^2 test.

3. Results

The genotype distributions of rs1057868 at *POR*, rs182420 at *SULT2A1*, and rs12086634 at *HSD11B1* were similar in the PA and control groups (Table 1). Neither did they differ in subgroup analyses: between the PA subjects with pubic or axillary hair and controls, between the PA subjects with biochemical adrenarche (S-DHEAS≥1 μmol/L) and controls with S-DHEAS<1 μmol/L, or between the PA and control girls (data not shown). All variants were in Hardy–Weinberg equilibrium. No differences in the clinical or metabolic parameters were observed between the genotype groups of rs1057868 at *POR* in the control or PA children (data not shown). The minor C variant of rs182420 at *SULT2A1* was associated with higher serum SHBG level and a trend for lower DHEAS/DHEA ratio in the controls, and with higher total cholesterol in the PA children (Table 2). Plasma triglyceride concentration was lower in the control subjects carrying the minor G variant of rs12086634 at *HSD11B1* compared with those homozygotic for the major T variant. These associations remained significant after adjusting for weight-for-height or baseline serum insulin concentration (data not shown).

4. Discussion

The genetic variants rs1057868 at *POR*, rs182420 at *SULT2A1* and rs12086634 at *HSD11B1* were not associated with PA. None of these SNPs has been studied in PA before.

The minor T variant of rs1057868 at *POR* leads to decreased 17-hydroxylase and 17,20-lyase activities of CYP17A1 [3]. We thus hypothesized that this common variant could contribute to androgen concentrations in PA. No differences in steroid concentrations between the *POR* genotype groups were found, however, and the genotype distribution was similar in the PA and control subjects.

There was no difference in the genotype distribution of the rs182420 at *SULT2A1* between the PA and control children. In a previous study, the minor C variant of rs182420 at *SULT2A1*

Table 2 – Clinical characteristics of children with premature adrenarche (PA) and controls according to genotype groups of rs182420 polymorphism at *SULT2A1* and rs12086634 polymorphism at *HSD11B1*.

rs182420 at SULT2A1	Controls			PA subjects		
Mean (95% CI)	T/T	T/C & C/C	P*	T/T	T/C & C/C	P*
Number of subjects	64	33		42	31	
Age at examination (y)	7.6 (7.3, 7.8)	7.4 (7.1, 7.7)	.35**	7.3 (7.0, 7.6)	7.7 (7.4, 8.0)	.05**
Weight-for-height (%)	108 (96, 112)	104 (100, 109)	.26†	117 (112, 123)	111 (103, 118)	.14†
DHEA (nmol/L)‡	3.9 (3.4, 4.5)	4.4 (3.7, 5.3)	.19	6.9 (6.1, 7.9)	7.2 (5.9, 8.9)	.79
DHEAS (μmol/L)‡	0.9 (0.7, 1.0)	0.7 (0.6, 0.9)	.51	1.7 (1.4, 2.1)	1.8 (1.4, 2.2)	.59
Δ4-A (nmol/L)‡	1.4 (1.2, 1.6)	1.2 (1.0, 1.5)	.48	2.5 (2.1, 2.9)	2.4 (1.9, 2.9)	.12
Cortisol (nmol/L)‡	228 (208, 250)	227 (199, 258)	.91	232 (201, 267)	233 (194, 281)	.79
DHEAS/DHEA ratio‡	218 (188, 252)	171 (143, 203)	.06	248 (220, 281)	244 (216, 276)	.60
SHBG (nmol/L)	94 (87, 101)	116 (106, 126)	**.001**	80 (67, 92)	84 (72, 97)	.15
Cholesterol (mmol/L)	4.2 (4.0, 4.4)	4.3 (4.1, 4.5)	.58	4.0 (3.8, 4.2)	4.6 (4.3, 4.8)	**<.001**
Triglycerides (mmol/L)‡	0.60 (0.54, 0.66)	0.51 (0.45, 0.57)	**.05**	0.67 (0.59, 0.76)	0.57 (0.50, 0.66)	.07
HOMA-IR‡	0.9 (0.8, 1.0)	0.8 (0.7, 1.0)	.38	1.2 (1.0, 1.4)	1.0 (0.9, 1.2)	.11
rs12086634 at *HSD11B1*	Controls			PA subjects		
Mean (95% CI)	T/T	T/G & G/G	P*	T/T	T/G & G/G	P*
Number of subjects	65	32		43	30	
Age at examination (y)	7.5 (7.3, 7.7)	7.4 (7.1, 7.8)	.69**	7.4 (7.1, 7.8)	7.5 (7.2, 7.7)	.79**
Weight-for-height (%)	107 (103, 111)	106 (101, 110)	.82†	116 (110, 123)	112 (106, 118)	.42†
DHEA (nmol/L)‡	4.3 (3.7, 4.9)	3.9 (3.3, 4.5)	.53	7.2 (6.2, 8.3)	6.9 (5.7, 8.3)	.51
DHEAS (μmol/L)‡	0. 87 (0.74, 1.02)	0.74 (0.60, 0.93)	.35	1.84 (1.54, 2.20)	1. 60 (1.25, 2.05)	.27
Δ4-A (nmol/L) ‡	1.4 (1.2, 1.6)	1.3 (1.1, 1.6)	.87	2.5 (2.2, 2.9)	2.3 (1.8, 2.9)	.28
Cortisol (nmol/L)‡	230 (210, 252)	227 (197, 262)	.95	216 (185, 251)	259 (219, 307)	.11
SHBG (nmol/L)‡	93 (85, 102)	104 (95, 114)	.13	76 (67, 88)	67 (56, 82)	.28
Cholesterol (mmol/L)	4.3 (4.1, 4.5)	4.1 (3.8, 4.3)	.06	4.2 (4.0, 4.5)	4.3 (4.0, 4.5)	.97
Triglycerides (mmol/L)‡	0.61 (0.55, 0.68)	0.49 (0.44, 0.54)	**.013**	0.61 (0.53, 0.69)	0.66 (0.58, 0.74)	.45
HOMA-IR‡	1.10 (1.02, 1.19)	1.04 (0.93, 1.17)	.37	1.32 (1.21, 1.44)	1.27 (1.45, 1.42)	.61
$ISI^{\ddagger}_{comp}$	0.98 (0.89, 1.09)	1.10 (0.95, 1.28)	.24	0.74 (0.64, 0.85)	0.80 (0.68, 0.95)	.33

*Analysis of covariance (ANCOVA) with age and sex as covariates unless otherwise marked, **t test, †Mann–Whitney test, ‡log-transformed and presented as geometric mean (95% CI); log-transformation was performed if non-normal distribution was found in either study group (residual of the linear model analysis). DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; Δ4-A, androstenedione; SHBG, sex hormone binding globulin; HOMA-IR, homeostasis model assessment for insulin resistance; ISI_{comp}, insulin sensitivity index.

was associated with lower DHEAS concentrations in PCOS women [4]. Although we did not find significant differences in serum DHEA or DHEAS concentrations between the genotype groups, a trend towards a lower DHEAS/DHEA ratio with the minor C variant was observed in the controls. The minor C variant of rs182420 was associated with higher total cholesterol in the PA children. SULT2A1 may actually have a role in lipid metabolism; it converts bile acids to sulfated metabolites in liver [13]. In addition, the minor C variant was associated with higher SHBG concentrations in the controls. Despite the known correlation of circulating SHBG concentrations with insulin sensitivity, we did not find an association between this polymorphism and insulin sensitivity.

Previous studies have linked decreased HSD11B1 activity with reduced risk of MBS [6,14,15], whereas increased HSD11B1 activity has been associated with features of MBS [16,17]. Furthermore, the minor G variant of rs12086634 at *HSD11B1* with decreased enzyme activity has been associated with higher adrenal androgen concentrations in lean PCOS women [5], and together with another *HSD11B1* polymorphism, it seems to protect against MBS [6]. We found no difference in the genotype distribution of rs12086634 at *HSD11B1* between the PA and control children, although our previous studies showed increased prevalence of childhood MBS in this PA cohort [7]. However, the minor G variant of rs12086634 was associated with lower plasma triglyceride concentrations in the controls, which is in line with previous studies in genetically modified mice [14]. This association was not seen in the PA group, suggesting that other factors may overcome the protective effect of this variant against MBS features in these children.

Our PA group is well-defined and one of the largest investigated so far. However, still larger study groups are needed to confirm our negative findings. Furthermore, some statistically significant differences in biochemical variables in this study may be due to multiple testing errors and should be confirmed in an independent study setting. Another limitation is that we allowed the examination ages up to one year higher than the definition for PA, which could affect some biochemical findings.

In conclusion, none of the candidate gene variants studied was associated with PA, suggesting that they do not significantly contribute to PA in our Finnish population. The minor C variant of rs182420 at *SULT2A1* may contribute to cholesterol metabolism in postadrenarcheal children.

Authors' Contributions

Pauliina Utriainen collected the data, and was together with Saila Laakso responsible for the data analyses and writing the manuscript. Jarmo Jääskeläinen and Raimo Voutilainen contributed to the planning of the study, interpreting the results and writing the manuscript.

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Conflict of interest

No conflict of interests.

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