Original Research

Relationship between polymorphisms in the sulfotransferase SULT2A1 gene and dehydroepiandrosterone sulfate concentration in children

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Abstract

Dehydroepiandrosterone sulfate (DHEA-S) is the most abundant circulating steroid hormone in humans, and has important physiological effects. A relationship has been suggested between variations of DHEA-S concentration and polymorphisms in the gene encoding sulfotransferase (SULT2A1), an enzyme that catalyzes the formation of DHEA-S from DHEA. We have investigated the relationship between the single nucleotide polymorphisms (SNPs) rs2637125 and rs182420 in the SULT2A1 gene and plasma DHEA-S concentration in children at two different ages. The sample population comprised 981 healthy 6–8-year-olds and 792 12–16-year-old children. In total, 12–16-year-old boys homozygous for the rare allele of rs182420 (CC) showed significantly lower DHEA-S concentration than TC boys, and both (TC and CC) had lower levels than TT boys. In all, 12–16 -year-old boy carriers of the rare allele for the rs2637125 polymorphism also showed lower levels of DHEA-S than GG carriers. No differences were observed in DHEA-S concentrations across genotypes in 6–8-year-old children. Our data show an age-related association of polymorphisms in the SULT2A1 gene with lower DHEA-S, suggesting that these polymorphisms may affect DHEA-S concentration in adults.

Keywords: DHEA-S concentration, sulfotransferase (SULT2A1) polymorphisms, children

Experimental Biology and Medicine

Introduction

Dehydroepiandrosterone (DHEA) and its sulfated ester (DHEA-S) are the most abundant steroid hormones in human circulation, and produce a wide variety of physiological effects, including cardioprotection, antidiabetic effects and immune-enhancing and cancer-preventing properties.[1] Low plasma DHEA-S concentration are observed in cases of obesity and cardiovascular disease risk.[2] Unlike DHEA, which shows diurnal variation, DHEA-S concentration are stable and have a longer half-life, making them more clinically useful to measure.

DHEA sulfation, leading to the formation of the abundant circulating steroid DHEA-S, occurs through the action of the steroid sulfotransferase SULT2A1.[3] Variations in SULT2A1 expression have been associated with variation in DHEA-S concentration.[4] Studies in cohorts of twin subjects have demonstrated substantial polygenic genetic influences on DHEA-S concentration.[5] A meta-analysis aimed at identifying the genetic determinants of plasma DHEA-S concentration has reported the association of the single nucleotide polymorphism (SNP) rs2637125 in the SULT2A1 gene with variations in plasma DHEA-S concentrations in Caucasian women.[6] Another SNP in the SULT2A1 gene (rs182420) has also been associated with lower DHEA-S concentration in women with polycystic ovary syndrome (PCOS),[7] although this association was not confirmed in a recent study in prepubertal Finnish children.[8] Other than this study, no other study has analyzed these polymorphisms in children.

In our work, we analyzed the relationship of the SNPs rs2637125 and rs182420 in the SULT2A1 gene with plasma DHEA-S concentration in population-based samples of healthy children at two different ages.

Materials and methods

Subjects

The sample population included 1773 healthy schoolchildren, 981 between 6 and 8 years and 792 between 12 and 16 years, who participated in a cross-sectional study examining cardiovascular risk factors in Spain. Children were selected through random cluster-sampling of schools, and were stratified by sex and socioeconomic level (i.e. public versus private schools). All children

Table 1 DHEA-S concentration (μg/dL) according to genotype of rs2637125 polymorphism at SULT2A1 by age and sex

12–16-year-old boys				12–16-year-old girls			
GG (271)	**GA (93)**	**AA (4)**	***P***	**GG (292)**	**GA (101)**	**AA (7)**	***P***
168.9 ± 94.4	142.1 ± 90.7	98.1 ± 38.8	GG-GA* GG-AA$^{0.06}$	158.5 ± 96.7	149.9 ± 88.3	148.8 ± 97.1	ns
6–8-year-old boys				**6–8-year-old girls**			
GG (330)	**GA (140)**	**AA (14)**	***P***	**GG (307)**	**GA (124)**	**AA (19)**	***P***
34.6 ± 36.6	36.6 ± 37.5	32.8 ± 33.3	ns	38.7 ± 38.4	36.7 ± 43.2	30.0 ± 39.1	ns

DHEA-S, dehydroepiandrosterone sulfate; SULT2A1, sulfotransferase
Data shown as mean ± standard deviation
P: ANOVA, Games-Howell *post hoc* test
$^{*}P \leq 0.05$

Table 2 DHEA-S concentration (μg/dL) according to genotype of rs182420 polymorphism at SULT2A1 by age and sex

12–16-year-old boys				12–16-year-old girls			
TT (195)	**TC (137)**	**CC (43)**	***P***	**TT (225)**	**TC (141)**	**CC (51)**	***P***
173.2 ± 99.0	169.4 ± 93.2	123.6 ± 58.4	TT-CC** TC-CC*	164.7 ± 92.1	146.1 ± 90.1	152.2 ± 93.4	ns
6–8-year-old boys				**6–8-year-old girls**			
TT (280)	**TC (171)**	**CC (46)**	***P***	**TC (258)**	**TC (178)**	**CC (46)**	***P***
33.8 ± 35.1	35.4 ± 40.1	30.2 ± 29.2	ns	38.3 ± 40.2	35.6 ± 33.9	37.4 ± 55.6	ns

DHEA-S, dehydroepiandrosterone sulfate; SULT2A1, sulfotransferase
Data shown as mean ± standard deviation
P: ANOVA, Games-Howell *post hoc* test
$^{*}P \leq 0.01$; $^{**}P \leq 0.001$

reported by their parents as suffering from cardiovascular or nephrological diseases were excluded in order to avoid a possible skewing of the variables of interest. The study protocol complied with the Helsinki Declaration guidelines and was approved by the Clinical Research Ethics Committee of the IIS-Fundación Jiménez Díaz. Parents were required to provide written consent in order for their children to participate in the study.

DHEA-S measurement

Fasting (12 hours) venous blood samples were collected early in the morning. DHEA-S was determined by radioimmunoassay (RIA) using a commercial kit: DSL-3500 DHEA-S (Diagnostic Systems Laboratories, Inc, Webster, TX, USA).

SULT2A1 polymorphism determination

Genotyping of the polymorphisms rs182420 and rs2637125 in the SULT2A1 gene was carried out using custom allelic discrimination TaqMan® assays (ID C8716099-10 and C16267062-20, respectively, [Applied Biosystems, Foster City, CA, USA]). A 7500 Fast RealTime PCR System (Applied Biosystems) was used to make allelic discrimination calls.

Statistical analysis

Statistical analyses were carried out using the SPSS software package, version 9.0 (SPSS, Inc., Chicago, IL, USA). Allele frequencies were calculated by allele counting. Analysis of variance (Games-Howell *post hoc* test) was used to compare DHEA-S concentration among genotypes.

Results

As expected, DHEA-S concentration were much higher in 12–16-year-olds than in 6–8-year-old children (Tables 1 and 2).

The prevalence of the less common allele for the rs2637125 polymorphism (A) was 16% (27% heterozygous GA and 3% homozygous AA). Prevalence of the rare allele for the rs182420 polymorphism (C) was 27% (35% heterozygous TC and 9% homozygous CC).

In 12–16-year-old boys, concentration of DHEA-S were lower in carriers of the rare allele A for the rs2637125 polymorphism than in carriers of the GG genotype (Table 1). Presence of the minor allele C for the rs182420 polymorphism was also significantly associated with reduced DHEA-S concentration in 12–16-year-old boys, with CC boys having lower levels than TC boys and both being lower than TT boys (Table 2). In girls, differences between genotypes did not reach statistical significance. A significant difference ($P < 0.05$) was also observed when analyzing DHEA-S concentration in boys carriers of both SNPs ($n = 76$) with levels

in boys without any of the polymorphisms ($n = 166$); this difference did not reach statistical significance in girls.

No differences were observed in DHEA-S concentration across genotypes in 6–8-year-old children (Tables 1 and 2).

Discussion

Plasma DHEA-S concentration show an age-associated pattern, as high levels are seen immediately after birth and then fall markedly within months. These levels start to rise again between 6 and 10 years, so the phenomenon referred to as adrenarche is associated with this increase in DHEA-S concentrations and premature adrenarche has been associated with higher levels of DHEA-S. DHEA-S concentration growth continues until they reach their peak between 15 and 20 years.[9] Reflecting this age-related pattern, DHEA-S concentrations in our study were much higher in 12–16-year-olds than in 6–8-year-old children, with the former exhibiting levels closer to those of adults.

Recently, a study aiming to identify genes regulating plasma DHEA-S concentration has suggested that polymorphisms in the sulfotransferase SULT2A1 gene are significantly related to DHEA-S concentration in healthy subjects.[6] In our population, the less common alleles for SNPs rs2637125 and rs182420 in the SULT2A1 gene are associated with lower plasma DHEA-S concentrations in 12–16-year-old boys. The minor allele of the SNP rs2637125 has also been negatively correlated with DHEA-S concentrations in the meta-analysis by Zhai *et al.*[6] and the rs182420 polymorphism has been associated with lower DHEA-S concentration in women with PCOS.[7] The fact that the polymorphisms are associated with lower DHEA-S concentration could explain interindividual variations in DHEA-S concentrations in humans.

However, our findings also show that these polymorphisms do not affect DHEA-S concentration in prepubertal children. In a recent case-control study comprising 73 prepubertal children with premature adrenarche and 97 healthy controls,[8] the authors also failed to find any association between the rs182420 polymorphism and DHEA-S concentration. Our findings, which show no association of the studied SULT2A1 polymorphisms with DHEA-S concentration in prepubertal children while exhibiting a significant relation with plasma DHEA-S concentrations in adolescents, suggest that the polymorphisms in the SULT2A1 gene appear to affect the levels of plasma DHEA-S when these levels are close to those characteristic in adults. The high variability of DHEA-S in the two groups of children included in our study appears to constitute a limitation to our work, and further studies in postpubertal subjects are required to confirm the interpretation of our results.

The absence of a significant association between these SNPs and DHEA-S in girls, in whom DHEA-S concentration are lower than in boys, reinforces the interpretation of our data connecting the different effect of these polymorphisms with different levels of DHEA-S.

It has been previously reported that variations in SULT2A1 expression are associated with variation in DHEA-S concentration,[4] and that SNPs in the SULT2A1 gene are associated with expression levels of SULT2A1 in human liver tissues.[6] It therefore seems that the association of these SNPs with DHEA-S concentrations is related with the effect of the polymorphisms on SULT2A1 expression.

In conclusion, polymorphisms in SULT2A1 are not related to DHEA-S concentrations in children at 6–8 years of age, when DHEA-S concentration are still low, but are significantly associated with DHEA-S concentrations in adolescent boys, when DHEA-S concentration are increasing toward adult levels.

Author contributions: AG-A and LO carried out the study data collection and laboratory work, revised the manuscript, and approved the final the manuscript as submitted. CG designed and conducted the study, analyzed data, drafted the manuscript and approved the final version of the manuscript.

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