

# A *SULT2A1* genetic variant identified by GWAS as associated with low serum DHEAS does not impact on the actual DHEA/DHEAS ratio

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## Abstract

DHEA is the major precursor of human sex steroid synthesis and is inactivated via sulfonation to DHEAS. A previous genome-wide association study related the single nucleotide polymorphism (SNP) rs2637125, located near the coding region of DHEA sulfotransferase, *SULT2A1*, to serum DHEAS concentrations. However, the functional relevance of this SNP with regard to DHEA sulfonation is unknown. Using data from 3300 participants of the population-based cohort Study of Health in Pomerania, we identified 43 individuals being homozygote for the minor allele of the SNP rs2637125 (AA) and selected two sex- and age-matched individuals with AG and GG genotype ( $n=172$ ) respectively. Steroid analysis including measurement of serum DHEA and DHEAS was carried out by liquid chromatography/mass spectrometry, employing steroid oxime analysis for enhancing the sensitivity of DHEA detection. We applied quantile regression models to compare median hormone levels across *SULT2A1* genotypes. Median comparisons by *SULT2A1* genotype (AA vs AG and GG genotypes respectively) showed no differences in the considered hormones including DHEAS, DHEA, androstenedione, as well as cortisol and cortisone concentrations. *SULT2A1* genotype also had no effect on the DHEA/DHEAS ratio. Sex-stratified analyses, as well as alternative use of the *SULT2A1* SNP rs182420, yielded similar negative results. Genetic variants of *SULT2A1* do not appear to have an effect on individual DHEA and DHEAS concentrations or the DHEA/DHEAS ratio as a marker of DHEA sulfonation capacity.

## Key Words

- ▶ DHEAS
- ▶ steroids
- ▶ genome-wide association study
- ▶ genetics
- ▶ epidemiology

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## Introduction

DHEAS is the most abundant steroid in human circulation and represents the conjugated, nonactive excretion form

of DHEA, which acts as the universal sex steroid precursor and the major source of androgens in women

(Arlt *et al.* 1999). Serum DHEAS shows an age-related decline, and at 70 years of age, circulating DHEAS concentrations have diminished by 90% compared with the peak levels achieved at 20–30 years of age (Orentreich *et al.* 1984). This has led to widespread speculation about a causative role of DHEAS in the development of age-related diseases and longevity. A previous genome-wide association study (GWAS) comprising 14 846 individuals identified eight common single nucleotide polymorphisms (SNPs) that implicate nearby genes as independently associated with serum DHEAS concentrations (Zhai *et al.* 2011), providing much needed insight into the metabolism of DHEAS. Among the identified genes, *SULT2A1*, a specialized sulfotransferase that converts DHEA to DHEAS in the adrenal cortex and liver, is an obvious candidate gene for subsequent functional studies (Rainey *et al.* 2002). Previous studies have established that the relationship between conjugated DHEAS and unconjugated DHEA is defined by *SULT2A1* activity (Hammer *et al.* 2005) and that impairment of DHEA sulfonation results in low DHEAS and concurrent androgen excess with high DHEA and androstenedione levels (Noordam *et al.* 2009). But the clinical relevance of this candidate gene identified by GWAS with regard to individually meaningful prognostic, diagnostic, and therapeutic information is unknown. Therefore, we measured serum DHEAS, DHEA, and androstenedione concentrations in a subset of 215 individuals of the Study of Health in Pomerania (SHIP) follow-up cohort to investigate potential differences in adrenal androgens, including the DHEA/DHEAS ratio, related to *SULT2A1* genotypes.

## Materials and methods

### Study population

The SHIP is a population-based cohort study conducted in the northeastern area of Germany. Details on the SHIP study design, recruitment, and procedures have been published previously (Völzke *et al.* 2011). The net sample drawn for the SHIP comprised 6265 eligible individuals (3160 women) with German citizenship and main residency in the study area, whereof 4308 (2192 women) aged 20–80 years finally participated in the baseline study (SHIP-0) between 1997 and 2001 (response 68.8%). During the 5-year follow-up (SHIP-1) between 2002 and 2006, 3300 (1711 women) participants were reexamined (response proportion 83.6%). All participants gave informed written consent. A review board of independent scientists monitored the study. The study protocol is

consistent with the principles of the Declaration of Helsinki, as reflected by an *a priori* approval of the Ethics Committee of the University of Greifswald. To assess the differences in adrenal androgens related to *SULT2A1* genotype, we identified 43 individuals homozygote for the minor allele of SNP rs2637125 (AA), additionally selected two sex- and age-matched individuals having AG and GG genotype ( $n=86$  each) respectively, and finally measured adrenal androgens in the serum of these 215 participants of the follow-up study SHIP-1.

Anthropometric measurements were taken and BMI was calculated from the body weight in kilogram and height in meters according to the formula:  $\text{kg/m}^2$ . A summative cardiovascular disease (CVD) score was built upon diagnosis of angina pectoris, peripheral artery disease, heart failure, stroke, or myocardial infarction. HbA1c concentrations were determined by HPLC (Bio-Rad Diamat). Diabetes mellitus was defined based on self-reported physicians' diagnosis or use of antidiabetic medication (ATC code A10) in the last 7 days, or HbA1c concentrations  $\geq 6.5\%$ . All blood samples had been drawn from the cubital vein between 0800 and 1800 h with participants in the supine position, and serum aliquots had been stored at  $-80^\circ\text{C}$  until analysis.

### Steroid analysis

Steroids were measured by liquid chromatography/tandem mass spectrometry (LC–MS/MS) employing a Waters Xevo mass spectrometer with Acquity uPLC system, fitted with a HSS T3, 1.8  $\mu\text{m}$ , 1.2  $\times$  50 mm column. LC–MS/MS conditions were an electrospray ionization source with capillary voltage 4.0 kV, a source temperature of 150  $^\circ\text{C}$ , and a desolvation temperature of 500  $^\circ\text{C}$ . Steroid oxime analysis, which facilitates enhanced detection, i.e. increased sensitivity, by formation of oxime derivatives of the steroid oxo-groups (Kushnir *et al.* 2006), was employed for the measurement of DHEA and androstenedione and carried out in positive mode, whereas DHEAS measurement was performed in negative mode. DHEA, androstenedione, cortisol, and cortisone were extracted from 150  $\mu\text{l}$  serum via liquid–liquid extraction using 1 ml tert-butyl-methyl-ether followed by derivatization into steroid oximes employing 100  $\mu\text{l}$  derivatization mixture (0.16 g hydroxylamine in 8 ml pyridine). For protein precipitation and extraction of DHEAS, 20  $\mu\text{l}$   $\text{ZnSO}_4$  0.1 mM and 100  $\mu\text{l}$  acetonitrile were added to 20  $\mu\text{l}$  serum before evaporation under constant flow of nitrogen. All steroids were separated using an optimized gradient system consisting of methanol with 0.1% formic acid and

quantified referring to a linear calibration series with appropriate internal standards, ranging from 0.1 to 250 ng/ml for steroid oxime analysis and 250 to 100 000 ng/ml for DHEAS analysis. Each steroid was identified by matching retention times and two mass transitions in comparison to a deuterated reference compound.

### Statistical analyses

Continuous data are expressed as median (25th and 75th percentiles) and quantitative data as percentage. Given the broad age range of our study population, we performed linear regression analyses modeling continuous age and DHEA/DHEAS ratio variables, but without any indication of a potential age dependency. Individuals being homozygote for the rs2637125 minor allele (AA) were used as reference group (AG vs AA and GG vs AA). Alternatively, we also modeled the *SULT2A1* SNP rs182420 with homozygous minor alleles (TT) as reference group (Goodarzi *et al.* 2007). Quantile regression models were used to compare median levels and calculate pairwise *P* values for continuous variables. Differences in categorical variables across allele pattern were assessed by pairwise  $\chi^2$  tests. To detect potential sex-specific differences, we repeated our analyses separately for men and women. All statistical analyses were performed using STATA 11.0 (Stata Corporation, College Station, TX, USA).

### Results

Comparing median hormone levels by *SULT2A1* genotype (AA vs AG and GG genotypes respectively), the conducted

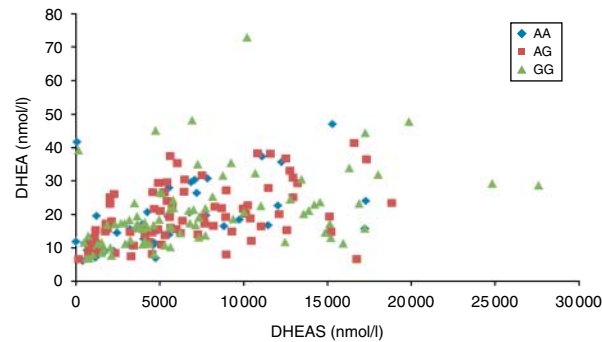
quantile regression models did not show any significant differences in the considered hormones including DHEA, DHEAS, cortisol, cortisone, or androstenedione concentrations (Table 1). *SULT2A1* genotype also had no impact on the DHEA/DHEAS ratio. There were also no differences with regard to age, BMI, smoking, CVD, or diabetes mellitus. We found a borderline significant (*P* value=0.037) difference between AA and GG genotypes with regard to cortisol concentrations (Table 1). A scatterplot of the actual DHEA and DHEAS concentration by *SULT2A1* genotype also failed to reveal clearly separated groups (Fig. 1). Analyses of the alternative *SULT2A1* SNP rs182420 showed comparable negative results as for the main analysis, with similar borderline significant differences in cortisol concentrations (*P* value=0.044) between TT and CC genotypes. Finally, sex-specific analyses of both *SULT2A1* SNPs revealed a significantly higher DHEA/DHEAS ratio among men with *SULT2A1* SNP rs2637125 AA genotype compared with men with AG (*P* value=0.006) and GG (*P* value=0.019) genotype. As we identified no differences in blood sampling time between the groups, co-incident group-specific variations in sampling time could be ruled out to explain this finding as a consequence of the diurnal rhythm of cortisol secretion.

### Discussion

The present post-GWAS functional study revealed no significant differences in DHEA or DHEAS concentrations with regard to *SULT2A1* genotype, a potential candidate gene predictive of low serum DHEAS concentrations

**Table 1** Characteristics of the study population by *SULT2A1* genotype (rs2637125). Values shown are expressed as median (25th and 75th percentiles) or percentage. The maximum number of observations was used for each measurement: DHEA: *n*=214, DHEAS: *n*=204, DHEA/DHEAS ratio: *n*=204, cortisol and cortisone: *n*=213. We used quantile regression models to compare median levels and calculate pairwise *P* values for continuous variables and pairwise  $\chi^2$  tests for categorical variables

Variable	AA ( <i>n</i> =43)	AG ( <i>n</i> =86)	AG vs AA <i>P</i> value	GG ( <i>n</i> =86)	GG vs AA <i>P</i> value
Age (years)	49.0 (40.0, 72.0)	50.0 (43.0, 72.0)	0.867	49.5 (42.0, 72.0)	0.989
DHEA (nmol/l)	16.6 (11.4, 22.6)	17.3 (11.9, 25.2)	0.759	17.3 (12.9, 23.3)	0.772
DHEAS (nmol/l)	4767 (2467, 7841)	5607 (2337, 10 116)	0.417	5735 (3601, 10 689)	0.320
DHEA/DHEAS ratio (nmol/l)	0.004 (0.002, 0.005)	0.003 (0.002, 0.006)	0.506	0.003 (0.002, 0.005)	0.446
Cortisol (nmol/l)	81.9 (51.1, 122.9)	89.8 (64.4, 142.5)	0.593	108.7 (65.7, 143.9)	0.037
Cortisone (nmol/l)	31.8 (23.6, 44.7)	38.9 (30.0, 53.1)	0.189	41.1 (29.2, 52.5)	0.071
Androstenedione (nmol/l)	1.6 (1.1, 2.5)	2.4 (1.1, 3.6)	0.162	2.0 (1.3, 3.4)	0.521
Blood sampling time (24 h)	11:00 (9, 12)	12:00 (10, 13)	0.450	11:00 (10, 13)	0.999
Current smoker (%)	37.2	30.2	0.173	30.2	0.379
BMI (kg/m <sup>2</sup> )	26.9 (24.5, 29.8)	26.7 (24.7, 29.3)	0.815	27.9 (25.1, 32.2)	0.269
Cardiovascular disease (%)	18.6	16.5	0.762	17.4	0.871
Diabetes mellitus (%)	11.6	9.3	0.679	9.3	0.679



**Figure 1**

Concentrations of DHEA to DHEAS by *SULT2A1* genotype. Individuals with AA genotype ( $n=39$ ) were compared with sex- and age-matched individuals carrying the AG genotype ( $n=83$ ) and GG genotype ( $n=82$ ) of the SNP rs2637125, all drawn from the SHIP follow-up study ( $n=3300$ ).

identified by a previous GWAS in 14 846 Caucasian individuals (Zhai *et al.* 2011).

*SULT2A1*, also termed DHEA sulfotransferase, is a specialized sulfotransferase that catalyzes the sulfonation of steroids and detoxification of bile acids in the liver and adrenal glands (Strott 2002). In this study, we were not able to detect any differences in median DHEA and DHEAS concentrations across the different *SULT2A1* genotype groups. Furthermore, *SULT2A1* genotype showed no impact on the conversion of DHEA into DHEAS, as indicated by the lack of a significant difference between the group-specific DHEA/DHEAS ratio, previously established as a valid surrogate marker for DHEA sulfonation capacity (Arlt *et al.* 2006, Noordam *et al.* 2009). These findings are in line with previous studies lacking any impact of *SULT2A1* allelic variants on serum adrenal hormones among African-American men (Wilborn *et al.* 2006), Finnish prepubertal children (Utriainen *et al.* 2012), or white women with polycystic ovary syndrome (PCOS; Goodarzi *et al.* 2007). The latter study found only one particular SNP (rs182420) within the *SULT2A1* gene related to lower DHEAS concentrations in women with PCOS, but not in women without PCOS (Goodarzi *et al.* 2007). Similarly, we were not able to detect an association of *SULT2A1* SNP rs182420 with DHEA or DHEAS concentrations among men or women from the general population.

Although the applied SNP rs2637125 was not the most strongly ( $P=2.61 \times 10^{-19}$ ) associated with DHEAS concentrations in the previous GWAS (the strongest hit was rs11761528 on *ZKSCAN5* with  $P=3.15 \times 10^{-36}$ ), it was located only 12 kb downstream of the functionally very relevant protein-coding gene *SULT2A1* (Zhai *et al.* 2011). However, potential explanations for the absent differences

in the actual DHEA and DHEAS concentrations with regard to *SULT2A1* genotype might relate to the unknown impact of this SNP on *SULT2A1* transcription and expression. Previous studies examining regulatory mechanisms of *SULT2A1* mRNA expression and enzymatic activity identified several factors as potent inhibitors of *SULT2A1* expression (McAllister & Hornsby 1988, Fang *et al.* 2007). For example, cytokines such as transforming growth factor- $\beta$  (TGF- $\beta$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) have been found to decrease adrenal cell *SULT2A1* mRNA levels (Parker *et al.* 1998). Decreased *SULT2A1* activity was also observed during the acute-phase response in a time- and dose-dependent manner in a murine model (Kim *et al.* 2004) and in patients with acute sepsis (Arlt *et al.* 2006). Also the transcriptional regulation via transcription factors SF-1 and GATA-6 might be an important modulator of *SULT2A1* expression and the production of adrenal DHEAS (Saner *et al.* 2005). In light of these limitations, the present data (although demonstrating no impact of *SULT2A1* genotype on actual DHEA and DHEAS concentrations) could not ultimately rule out a potential influence of *SULT2A1* on mechanisms regulating DHEAS production in the human adrenal.

Asking what GWAS will mean to the clinical endocrinologist, the present post-GWAS functional study puts further weight on the notion that candidate genes identified by GWAS do not necessarily translate into clinically meaningful prognostic, diagnostic, and therapeutic information for individuals (McCarthy 2009). However, given the generally small proportion of inter-individual variability in disease predisposition explained by single genetic variants (Maher 2008), GWAS could only be a first step and essential starting point for the molecular classification of disease subtypes and the subsequent translation into novel therapeutic and preventative approaches (Haring & Wallaschofski 2012).

#### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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