



# Allopregnanolone levels and depressive symptoms during pregnancy in relation to single nucleotide polymorphisms in the allopregnanolone synthesis pathway



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## ABSTRACT

Allopregnanolone, a neurosteroid whose levels rise throughout gestation, putatively stabilizes antenatal mood. The present study aimed to investigate associations of plasma allopregnanolone to antenatal depressive symptoms, as well as to genetic and obstetric factors.

Allopregnanolone plasma levels from 284 pregnant women were measured around gestational week 18. Haplotype tag single nucleotide polymorphisms in the aldo-keto reductase family 1, members C2 and C4 (*AKR1C2*, *AKR1C4*), and steroid 5 alpha-reductase 1 and 2 (*SRD5A1*, and *SRD5A2*) genes were genotyped in a larger sample of pregnant women ( $n = 1351$ ). The Edinburgh Postnatal Depression Scale (EPDS) was administered via web-questionnaires in gestational weeks 17 and 32. Demographic and obstetric data was retrieved from web-questionnaires and medical records.

There was no association between allopregnanolone levels and depressive symptoms. Furthermore, no associations between allopregnanolone level and synthesis pathway genotypes were found after accounting for multiple comparisons. However, exploratory analyses suggested that the women who were homozygous for the minor allele of the *AKR1C2* polymorphism rs1937863 had nominally lower allopregnanolone levels and lower depression scores in gestational week 17, but also the highest increase in depression scores between week 17 and 32. Additionally, higher body mass index was associated with lower allopregnanolone levels.

The results do not support second trimester plasma allopregnanolone as a mood stabilizing factor. However, we speculate that *AKR1C2* variation may alter the susceptibility to depressive symptoms through effects on central allopregnanolone synthesis. Another implication of this study is that the relationship between neuroactive steroids and obesity in pregnancy deserves to be investigated.

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## 1. Introduction

Allopregnanolone is a neuroactive steroid which increases around 25 times in the maternal circulation during pregnancy (Kancheva et al., 2007). Allopregnanolone enhances signaling at  $\gamma$ -aminobutyric acid type A ( $GABA_A$ )-receptors and the serum concentrations found in pregnancy are within the range associated with sedative and anxiolytic effects when administered to non-pregnant women (Timby et al., 2006). The high concentration during pregnancy is likely to be of importance for central nervous system functions in both mother and fetus.

In rodent pregnancy, allopregnanolone specifically suppresses the maternal hypothalamic-pituitary-adrenal (HPA-) axis response to stress through increased opioid and GABAergic inhibition of corticotropin-releasing hormone (CRH) release (Brunton, 2016). One

example of how this mechanism may protect the fetal nervous system is that experimentally lowered allopregnanolone exposure during fetal life increases anxiety-like behavior in guinea pigs (Cumberland et al., 2017). Human pregnancy also brings about a suppression of the maternal stress response, which is thought to be important for optimal fetal development, especially the regulation of the fetal HPA-axis (Brunton, 2016). A reduction of the maternal stress response may also provide a balance against the pregnancy-related endocrinological and psychosocial strains on maternal mood.

Allopregnanolone has also been associated with mood in humans. For example, patients with major depression have been reported to have low levels of serum and cerebrospinal fluid allopregnanolone compared to healthy controls (Romeo et al., 1998; Strohle et al., 1999; Uzunova et al., 1998), while SSRI treatment has been reported to increase allopregnanolone levels in clinical populations (Romeo et al., 1998; Uzunova et al., 1998). In line with earlier observations, we have previously found lower serum allopregnanolone to be associated with

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depressive symptoms in late pregnancy (Hellgren et al., 2014) but allopregnanolone levels in SSRI treatment during pregnancy have not been reported.

Allopregnanolone is a metabolite of progesterone, and is produced in the corpus luteum and the adult adrenal (Genazzani et al., 2002; Ottander et al., 2005). During pregnancy, the placental synthesis produces maternal serum progesterone levels which are over 30 times higher than before pregnancy (Kancheva et al., 2007). Progesterone, in turn, is reduced into 5 $\alpha$ -dihydroprogesterone (DHP) by 5 $\alpha$ -reductases in the liver and in the placenta (Milewich et al., 1979). 5 $\alpha$ -DHP can then be metabolized to allopregnanolone by 3 $\alpha$ -hydroxysteroid dehydrogenases (HSD), mainly in the liver (Penning et al., 2000). Importantly, allopregnanolone is also synthesized de novo within the brain (Penning et al., 2000).

The three human 5 $\alpha$ -reductases, types 1–3, are encoded by the *SRD5A1*, *SRD5A2*, and *SRD5A3* genes. In the brain, 5 $\alpha$ -reductase 1 is the predominant isoform (Stoffel-Wagner et al., 2000; Stoffel-Wagner et al., 1998), whereas both 5 $\alpha$ -reductase 1 and 2 are present in the adult liver and placenta (Thigpen et al., 1993; Vu et al., 2009). The 5 $\alpha$ -reduction of progesterone is thought to be the rate limiting step in the conversion of progesterone to allopregnanolone (Dong et al., 2001). There are four human 3 $\alpha$ -HSDs, type 1 – type 4, encoded by the genes *AKR1C4* - *AKR1C1*. All are expressed in human liver, but *AKR1C1* and *AKR1C2* are the major isoforms in the human brain (Penning et al., 2000). It is not known whether existing variations in these genes affect central or peripheral allopregnanolone production, but they may influence aspects of anxiety and mood (Johansson et al., 2011; Quast et al., 2014).

Given the potential of maternal allopregnanolone to affect the maternal and fetal central nervous systems, it is of interest to explore which maternal factors are associated with allopregnanolone levels during pregnancy. Aside from the aforementioned involvement of allopregnanolone in stress and mood regulation, there is evidence to support that allopregnanolone is decreased in non-pregnant smokers (Dušková et al., 2012). Regarding obstetric factors, Luisi et al. (2000) reported higher allopregnanolone throughout pregnancy in five women with chronic hypertension and pre-eclampsia than in controls. Another finding that implicates metabolic factors is that circulating late pregnancy allopregnanolone is lower in women with larger pregnancy weight gain (Lundqvist et al., 2017).

In this study we aimed to extend our previous results of lower circulating levels allopregnanolone during late pregnancy in women with antenatal depressive symptoms (Hellgren et al., 2014), by examining mid-pregnancy levels of allopregnanolone in relation to repeated depression ratings. We also aimed to investigate whether genetic variation in the maternal genes *AKR1C2*, *AKR1C4*, *SRD5A1*, and *SRD5A2*, all coding for enzymes in the synthesis pathway from progesterone to allopregnanolone, account for any of the interindividual variation in allopregnanolone levels. Extending the hypothesis that high allopregnanolone is a mood stabilizing factor during pregnancy, we anticipated that genotypes associated with lower allopregnanolone levels, and thus presumably with lower synthesis throughout pregnancy, should entail higher incidence of depressive symptoms.

Furthermore, we aimed to do exploratory analyses of associations between allopregnanolone levels and maternal and obstetric variables previously found to correlate with allopregnanolone, and/or constituting possible confounders.

## 2. Material and methods

### 2.1. Setting

This study comprises two overlapping populations combined from two different studies at the Department of Women's and Children's Health, Uppsala University, and Uppsala University hospital: the BASIC (Biology, Affect, Stress, Imaging, Cognition) study and the Uppsala

Biobank of Pregnant Women. Subjects in both populations participated in the BASIC study, through which they provided longitudinal data on mood during pregnancy. Plasma allopregnanolone data for population 1 were obtained from the Uppsala Biobank of Pregnant Women, while blood samples for the genetic analyses (population 2) were collected within the BASIC study. Both studies have been approved by the Regional Ethical Review Board of Uppsala, Sweden. All study procedures were in accordance with ethical standards for human experimentation.

### 2.2. The BASIC study

The BASIC study is a population-based, longitudinal study of psychological wellbeing during pregnancy and the postpartum period in Uppsala County, Sweden. All women attending the routine ultrasound examination are invited to participate in the study. Exclusion criteria for the BASIC study are: inability to adequately communicate in Swedish, confidential personal data, pathologic pregnancies as diagnosed by routine ultrasound (miscarriages or malformations leading to termination of pregnancy), and age under 18 years. Participating women complete web-based questionnaires containing questions on demographic variables, prior psychiatric history, ongoing medication, and the Swedish version of the Edinburgh Postnatal Depression Scale (EPDS) (Cox et al., 1987; Wickberg and Hwang, 1996) at gestational week 17 and 32. An EPDS score of 13 or more during pregnancy has been found to render optimal sensitivity and specificity with a positive predictive value of 54% for an ongoing depressive episode (Rubertsson et al., 2011), and has been used as the cut-off in our analysis. In addition to answering web-questionnaires, the majority of BASIC participants also consent to donate blood samples at delivery. Venous blood samples are collected in EDTA-containing tubes. The samples are separated by centrifugation and buffy coat was stored at  $-70^{\circ}\text{C}$  until analysis.

### 2.3. The Uppsala Biobank of Pregnant Women

Blood samples for the determination of allopregnanolone (population 1) were collected as part of the ongoing population-based Uppsala Biobank of Pregnant Women. Invitation to participate in the biobank is done at random, when a research nurse is available at the ultrasound unit. Approximately 30% decline participation and it is estimated that the biobank covers approximately half of the pregnant population of Uppsala County (Granfors et al., 2013). Included women are 18 years or older, Swedish-speaking, and free from blood-borne disease (HIV, hepatitis C and hepatitis B). Upon inclusion, brief demographic data are collected, including ongoing chronic disorders, ongoing medication, smoking, height, and first trimester weight. Venous blood samples are collected in conjunction with the routine ultrasound screening around gestational week 18. The samples are separated by centrifugation within 2 h of sampling and plasma samples are stored at  $-70^{\circ}\text{C}$  until analysis.

### 2.4. Population 1

Among the BASIC participants who had donated a blood sample (between June 2008 and January 2013) to the Uppsala Biobank of Pregnant Women, we included 284 women for allopregnanolone analysis

**Table 1**

Characteristics of population 1, continuous variables representing important maternal and obstetric data, and potential confounders. Correlation coefficients (Pearson's  $r$ ) with maternal serum allopregnanolone are provided.

| Variable (n)                                 | Mean $\pm$ SD  | $r$   | $p$    |
|--|----------------|-------|--------|
| Age, years (284)                             | 30.1 $\pm$ 4.7 | −0.08 | 0.2    |
| First trimester BMI, kg/m <sup>2</sup> (284) | 24.2 $\pm$ 4.6 | −0.34 | <0.001 |
| Gestational age at sampling, weeks (284)     | 18.0 $\pm$ 1.0 | 0.14  | 0.015  |
| Gestational age at delivery, weeks (284)     | 40.0 $\pm$ 1.6 | −0.01 | 0.8    |
| Infant birth weight, g (279)                 | 3652 $\pm$ 544 | 0.04  | 0.5    |

**Table 2**  
Characteristics of population 1, categorical variables representing important maternal and obstetric data, and potential confounders. Comparisons (*t*-tests) of maternal serum allopregnanolone across groups are provided. In case of missing values percentages are given in relation to valid responses.

|   | n (%)     | Serum allopregnanolone (nmol/l), mean ± SD | <i>p</i>            | <i>d</i> <sup>a</sup> | Adj. <i>p</i> <sup>a</sup> |
|---|-----------|--|---------------------|-----------------------|----------------------------|
| All   | 284       | 18.3 ± 6.4                                 |                     |                       |                            |
| Educational level   |           |  | 0.3                 | 0.13                  |                            |
| ≤ 12 years  | 83 (29%)  | 17.7 ± 6.5                                 |                     |                       |                            |
| > 12 years  | 201 (71%) | 18.5 ± 6.3                                 |                     |                       |                            |
| Parity  |           |  | 0.3                 | 0.13                  |                            |
| Primipara   | 152 (54%) | 18.6 ± 6.5                                 |                     |                       |                            |
| Multipara   | 132 (46%) | 17.9 ± 6.3                                 |                     |                       |                            |
| Infant sex  |           |  | 0.5                 | 0.08                  |                            |
| Female  | 135 (48%) | 18.1 ± 6.7                                 |                     |                       |                            |
| Male  | 147 (52%) | 18.5 ± 6.1                                 |                     |                       |                            |
| SSRI treatment during pregnancy                             |           |  | 0.3                 | 0.25                  |                            |
| Yes   | 21 (7%)   | 16.8 ± 6.4                                 |                     |                       |                            |
| No  | 263 (93%) | 18.4 ± 6.4                                 |                     |                       |                            |
| Smoking during 1st trimester                                |           |  | 0.007 <sup>b</sup>  | 0.72                  | 0.050                      |
| Yes   | 15 (5%)   | 14.0 ± 4.2                                 |                     |                       |                            |
| No  | 269 (95%) | 18.5 ± 6.4                                 |                     |                       |                            |
| First trimester BMI > 30 kg/m <sup>2</sup>                  |           |  | >0.001 <sup>c</sup> | 0.75                  | >0.001                     |
| Yes   | 32 (11%)  | 14.1 ± 5.9                                 |                     |                       |                            |
| No  | 252 (89%) | 18.8 ± 6.3                                 |                     |                       |                            |
| Pregnancy induced hypertension (without pre-eclampsia)      |           |  | 0.037 <sup>d</sup>  | 0.50                  | 0.4                        |
| Yes   | 19 (7%)   | 15.3 ± 5.1                                 |                     |                       |                            |
| No  | 265 (93%) | 18.5 ± 6.4                                 |                     |                       |                            |
| Pre-eclampsia   |           |  | 0.5                 | 0.23                  |                            |
| Yes   | 8 (3%)    | 21.0 ± 12.4                                |                     |                       |                            |
| No  | 276 (97%) | 18.2 ± 6.2                                 |                     |                       |                            |
| Low depression ratings, EPDS < 13 week 17 and 32            | 202 (71%) | 17.9 ± 6.4                                 |                     |                       |                            |
| One high depression rating, EPDS score ≥ 13 week 17 or 32   | 59 (21%)  | 18.8 ± 6.8                                 | 0.4 <sup>c</sup>    | 0.13                  | 0.6 <sup>d</sup>           |
| Two high depression ratings, EPDS score ≥ 13 week 17 and 32 | 23 (8%)   | 20.0 ± 5.6                                 | 0.1 <sup>c</sup>    | 0.32                  | 0.3 <sup>d</sup>           |

<sup>a</sup> Cohen's *d*.

<sup>b</sup> Adjusted for first trimester BMI and gestational age at sampling.

<sup>c</sup> Compared with women with low depression ratings during pregnancy (EPDS < 13 in gestational week 17 and 32).

<sup>d</sup> Adjusted for BMI and gestational age at sampling, and excluding women on SSRIs.

(population 1). Population 1 consisted of 82 women with depressive symptoms (EPDS ≥ 13 in week 17 and/or 32) and 202 controls (EPDS scores of 12 or less at both time-points). In an attempt to differentiate between milder and more severe or more persistent symptoms we further separated the cases in two groups according to whether they rated above the cut-off on one (*n* = 59) or two (*n* = 23) time-points during pregnancy. Relevant background and obstetric data was retrieved from web-questionnaires and medical records. Participant characteristics of population 1 are reported in Table 1 (continuous) and Table 2 (categorical). Among the women in population 1, 212 were also part of population 2 and thus genotyped for allopregnanolone synthesis SNPs (Supplementary Fig. 1).

## 2.5. Population 2

Blood samples for genotyping (population 2) were obtained from the BASIC study. Blood samples were collected between January 2009 and March 2013. Relevant background and obstetric data was retrieved from web-questionnaires and medical records. The women included in population 2 had self-reported Caucasian background and completed EPDS questionnaires from gestational week 17 and/or 32 (*n* = 1351), and population characteristics are given in Table 3.

## 2.6. Allopregnanolone analyses (population 1)

Allopregnanolone plasma concentrations were determined by Umeå Neurosteroid Research Center, as previously described (Timby et al., 2006). Briefly, plasma allopregnanolone was measured using radioimmunoassay (RIA) after extraction with diethyl ether and purification by celite chromatography to reduce cross reactivity. The antibody used was raised against 3α-hydroxy-20-oxo-5α-pregnan-11-yl carboxymethyl ether coupled with bovine serum albumin as antigen

(AgriSera AB, Umeå, Sweden). All samples were counted in a RackBeta (Wallace, Finland) scintillation counter.

## 2.7. Genetic analyses (population 2)

DNA was isolated from blood using the silica-based Kleargene DNA extraction method (KBioscience®). Four candidate genes involved in the allopregnanolone synthesis pathway were selected: aldo-keto reductase family 1, member C2 (*AKR1C2*); aldo-keto reductase family 1, member C4 (*AKR1C4*); steroid-5-α-reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 1) (*SRD5A1*); and steroid-5-α-reductase, alpha polypeptide 2 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 2) (*SRD5A2*).

**Table 3**

Demographic characteristics of population 2. Normally distributed continuous data is presented as mean ± SD, skewed continuous data as median (inter quartile range, IQR), and categorical data as n (%). Percentages are given in relation to available data (total *n* = 1351).

|                         | Mean ± SD, median (IQR), or n (%) | n    |
|-------------------------|-----------------------------------|------|
| Age, years              | 31 ± 5 <sup>a</sup>               | 1349 |
| BMI, kg/m <sup>2</sup>  | 22.9 (21.1–25.6) <sup>b</sup>     | 1336 |
| Infant birth weight, g  | 3650 ± 530 <sup>a</sup>           | 1203 |
| Primiparity             | 565 (47%) <sup>c</sup>            | 1209 |
| Female infant sex       | 605 (47%) <sup>c</sup>            | 1299 |
| >12 years of education  | 1036 (77%) <sup>c</sup>           | 1348 |
| EPDS score week 17      | 5 (2–8) <sup>b</sup>              | 1334 |
| EPDS score week 32      | 5 (2–8) <sup>b</sup>              | 1304 |
| EPDS score ≥ 13 week 17 | 117 (9%) <sup>c</sup>             | 1334 |
| EPDS score ≥ 13 week 32 | 127 (10%) <sup>c</sup>            | 1304 |

<sup>a</sup> Mean ± SD.

<sup>b</sup> Median (IQR).

<sup>c</sup> n (%).

Gene data was obtained from HapMap database for a region spanning each gene  $\pm 100$  base pairs, and haplotype-tag SNPs were selected using Haploview 4.2 (minimum minor allele frequency of 0.1;  $r^2 \geq 0.8$ , pairwise tagging only). Candidate markers from previous association studies were also considered. A detailed description of the 46 selected polymorphisms as well as of allele and genotype frequencies is reported as supplementary material (Supplementary Table 1). Genotyping analysis was performed using a fluorescence-based competitive allele-specific PCR (KASPar) assay (KBioscience®). No-template control samples were included to enable the detection of contamination or non-specific amplification. The genotyping call rate was  $\geq 97\%$  in the whole sample. Five SNPs with a minor allele frequency below 0.05 were excluded along with 3 SNPs with genotypes which were not in Hardy-Weinberg Equilibrium. Therefore, 38 SNPs were included in the final analyses (Supplementary Table 1). Linkage disequilibrium and potential haplotype blocks were estimated with the EM algorithm using SNP & Variation Suite 8.4.1 (GoldenHelix®). The SNPs showed low linkage disequilibrium ( $r^2$ ) between each other (Fig. 1), and no haplotypes were computed. The genotype analyses were performed blindly with regard to psychosocial data.

2.8. Statistical analyses

Allopregnanolone concentrations were considered normally distributed (Shapiro Wilk  $> 0.95$ ). Associations with continuous variables were tested with Pearson's correlations, and group comparisons with

independent *t*-tests. Where the results warranted follow-up analyses, univariate ANOVAs were used to adjust for covariates.

For the genetic analyses, linear regression or F-test analyses were performed to evaluate the contribution of the markers to allopregnanolone plasma concentrations, within allelic (D vs. d), genotype (DD vs. Dd vs. dd), additive (dd  $>$  Dd  $>$  DD), dominant (DD/Dd vs. dd), and recessive (DD vs. Dd/dd) models. The same models were applied when comparing EPDS scores between genotype groups, but the number of analyzed SNPs was reduced to those which showed nominally significant differences in allopregnanolone level.

The IBM SPSS version 20 software was used for data analysis. Statistical significance was set at a *p*-value of  $< 0.05$ , with false detection rate (FDR) correction applied in the SNP-analyses to adjust for multiple testing. Effect sizes are reported as Cohen's *d* for pairwise comparisons,  $r/r^2$  for linear analyses, and  $\eta^2$  for ANOVAs. Unless otherwise specified, estimates are presented as mean  $\pm$  standard deviation.

3. Results

3.1. Allopregnanolone levels in relation to maternal and obstetric variables (population 1)

All women were married or co-habiting with a partner and no one reported alcohol use during the first trimester. Group characteristics are shown in Tables 1 and 2, together with the linear associations with allopregnanolone, and allopregnanolone plasma concentrations in relation to various group characteristics. Allopregnanolone was

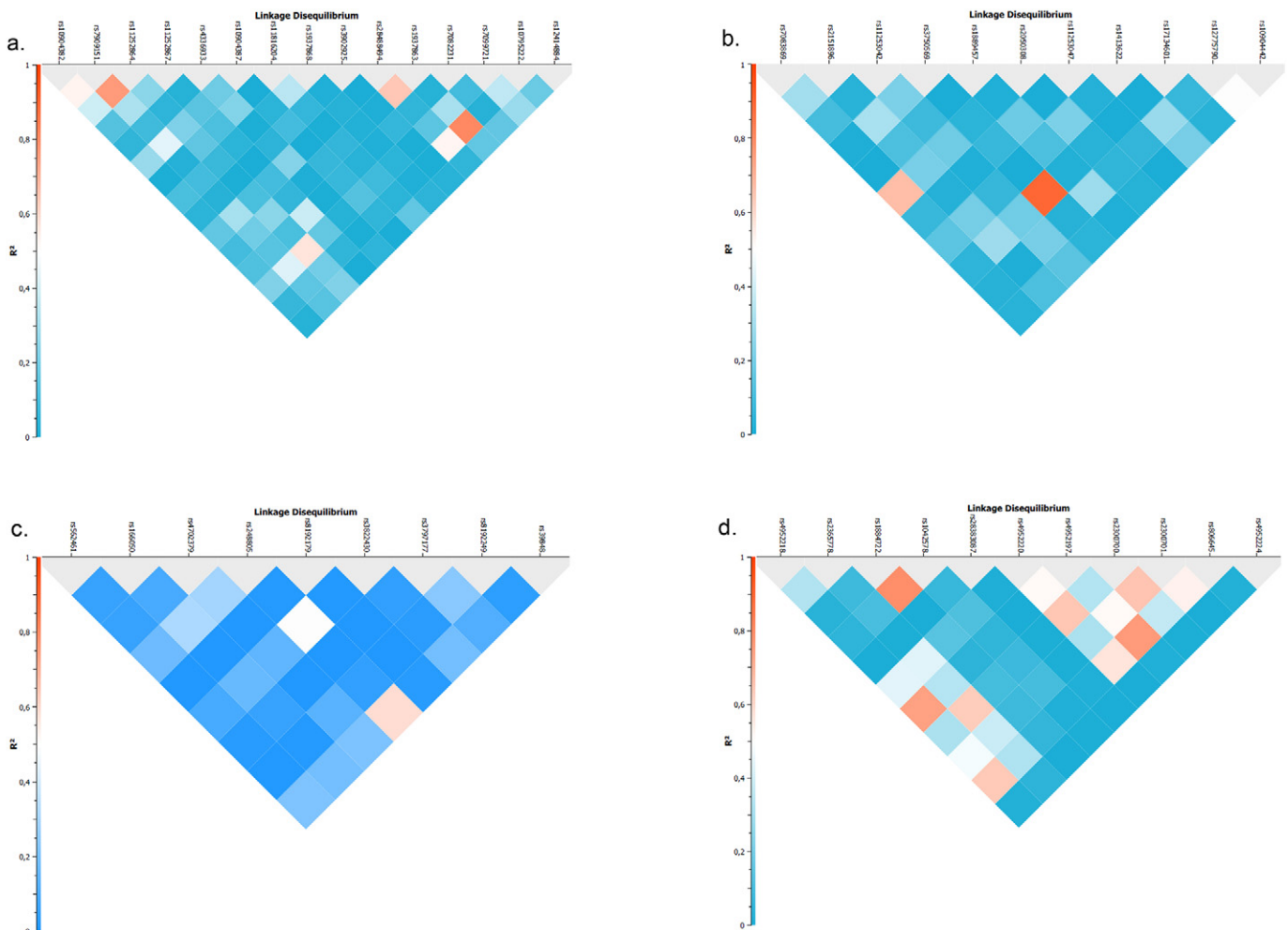


Fig. 1. Linkage disequilibrium plots of analyzed SNPs by gene: a) *AKR1C2* b) *AKR1C4* c) *SRD5A1* d) *SRD5A2*.

negatively correlated with first trimester body mass index (BMI) ( $r = -0.342$ ,  $p < 0.001$ , Fig. 2) and positively correlated with gestational age at blood sampling ( $r = 0.146$ ,  $p = 0.013$ , Table 1), both these correlations remained significant when combined in a follow-up ANOVA (data not shown). Women who smoked during the first trimester, and women who developed pregnancy-induced hypertension, respectively (Table 2), had significantly lower allopregnanolone than the rest of the population, but these associations did not remain when adjusted for BMI and gestational age at sampling.

### 3.2. Allopregnanolone in relation to depressive symptoms (population 1)

There was no group difference in allopregnanolone level between women with EPDS scores above and below the cut-off on none, one, or both of the time-points during pregnancy (Table 2). Neither were there any differences in allopregnanolone when considering high EPDS scorers in week 17 and 32 separately (data not shown). Excluding SSRI-users and adjusting for BMI and gestational age at sampling did not alter this result. Similarly, excluding women with pregnancy-induced hypertension, first trimester smoking, and BMI over 30 kg/m<sup>2</sup> did not alter the results (data not shown).

### 3.3. Allopregnanolone levels in relation to synthesis pathway SNPs (population 1 and 2)

Four SNPs showed nominally significant group differences in allopregnanolone concentration in at least one model, but no SNP survived correction for multiple testing (Table 4). The rs28488494 SNP of the *AKR1C2* gene was nominally significant in four out of five models (see Table 4), where women with the GG genotype had the highest mean allopregnanolone ( $19.1 \pm 6.2$  nmol/l,  $n = 129$ ), followed by the heterozygotes ( $17.0 \pm 6.1$ ,  $n = 72$ ), and the women with the AA genotype ( $15.8 \pm 5.5$ ,  $n = 11$ ). The rs1937863 (*AKR1C2*), rs806645, and rs2300701 (both *SRD5A2*) also showed nominally significant differences with a similar pattern of lower serum allopregnanolone associated with presence of the minor allele (Table 4). Group differences in allopregnanolone between genotypes for rs28488494 remained nominally significant when adjusted for BMI and gestational age at sampling ( $p = 0.04$ ), but not the other three SNPs ( $p = 0.05$ – $0.07$ ).

### 3.4. Depressive symptoms in relation to four allopregnanolone synthesis pathway SNPs with nominally significant associations to allopregnanolone level (population 2)

The results are summarized in Supplementary Table 2. Overall, the effect sizes of the nominally significant group differences in EPDS scores were small, and only the *AKR1C2* SNP rs1937863 survived correction for

multiple comparisons, in the allelic model of EPDS scores in gestational week 17, so that the minor allele was associated with slightly lower mean depression scores. An exploratory repeated measures ANOVA indicated that the women who were minor allele homozygotes in either of the two adjacent *AKR1C2* polymorphisms which were nominally associated with lower serum allopregnanolone level (rs1937863 and rs28488494) also had a significantly greater increase in EPDS score between week 17 and week 32 (time by genotype interaction for rs1937863:  $F(2,1292) = 5.8$ ,  $p = 0.003$ , partial  $\eta^2 = 0.009$ ; rs28488494:  $F(2,1289) = 3.3$ ,  $p = 0.039$ , partial  $\eta^2 = 0.005$ ), (Fig. 3). For the other two (*SRD5A1*) SNPs which were also nominally associated with allopregnanolone level (Table 4) there was no time by genotype interaction ( $p > 0.5$ ).

## 4. Discussion

We found no association between depressive symptoms during pregnancy and early second trimester allopregnanolone levels. This is not in line with our previous findings in late pregnancy, where low allopregnanolone levels were associated with more depressive symptoms (Hellgren et al., 2014). The different gestational age at sampling is one possible reason for this discrepancy. Allopregnanolone production continues to rise until term pregnancy (Luisi et al., 2000), and possibly does not reach a mood stabilizing level until then. Also, the potentially stressful blood sampling setting (routine ultrasound screening) can possibly have increased interindividual variation in plasma allopregnanolone and obscured any depression-related group differences. Allopregnanolone has been reported to correlate with state anxiety throughout pregnancy (Deligiannidis et al., 2016), and a decrease in serum allopregnanolone in response to laboratory stressors during second trimester of human pregnancy has been reported in a recent pilot study (Crowley et al., 2016). Nevertheless, no difference in allopregnanolone levels was found in a small number of clinically depressed pregnant women and controls (Pearson Murphy et al., 2001), and two larger longitudinal studies found no correlation between allopregnanolone and depression during pregnancy (Deligiannidis et al., 2016; Osborne et al., 2017). Thus, the most plausible conclusion is that a clinically significant association between peripheral allopregnanolone levels and mood during pregnancy does not exist, at least not during the second trimester. Thus, further study of allopregnanolone's potential role in mood during pregnancy likely requires access to central nervous system levels.

We found no effect of any of the investigated polymorphisms in synthesis pathway enzyme genes on the second trimester plasma allopregnanolone levels, after correcting for multiple comparisons. However, the levels are rapidly increasing during mid-pregnancy and in contrast to the momentary level of allopregnanolone at blood sampling, any functional genetic variations in the synthesis enzymes also have the potential to be associated with allopregnanolone and mood over time. Our exploratory analyses showed that rs1937863, one of the four SNPs which were in nominally significant association with allopregnanolone before correction, was associated with EPDS score in week 17. Contrary to our hypothesis, it was the women with the genotype with the lower mean allopregnanolone who had lower depression scores. Furthermore, the women with two copies of either of the minor *AKR1C2* alleles of rs1937863 and rs28488494, associated with the nominally lowest allopregnanolone levels, had a larger increase in depressive symptoms between week 17 and 32. This could suggest that an efficient conversion of progesterone to allopregnanolone, while not having obvious impact in mid-pregnancy, becomes an increasingly important mood balancing factor as the pregnancy progresses. Especially since the allopregnanolone level in mid-pregnancy is only around half of what it will be in late pregnancy (Hellgren et al., 2014). Although this preliminary SNP-mood-time interaction needs future replication before any firm conclusions can be drawn, we speculate that the effect on mood could also differ widely between trimesters due to factors

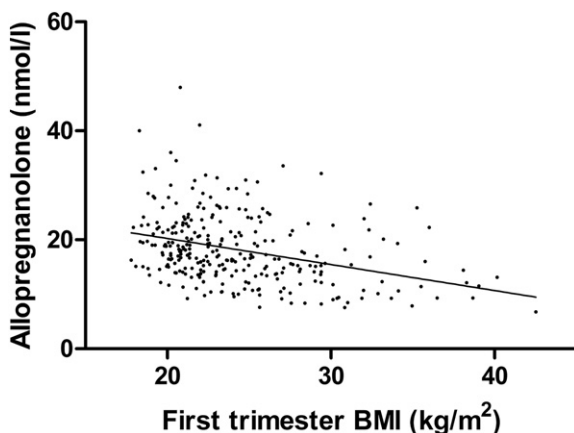


Fig. 2. Maternal serum allopregnanolone at 18 weeks gestation in relation to first trimester body mass index (BMI) ( $r = -0.34$ ,  $p < 0.001$ ).

**Table 4**

SNP markers with nominally significant associations to plasma allopregnanolone by statistic model (overlap of population 1 and 2). No marker remained significant after false discovery rate (FDR) correction (corrected  $\alpha = 0.00132$ ).

| Model                       | Marker     | Gene          | Mean group difference in allopregnanolone | n                      | Uncorrected statistics |                | Effect size |
|-----------------------------|------------|---------------|---|------------------------|------------------------|----------------|-------------|
|                             |            |               |   |                        | F-test p               | F-test F       | Cohen's d   |
| Allelic (D vs. d)           | rs2300701  | <i>SRD5A2</i> | −1.3 nmol/l                               | 209                    | 0.04                   | 4.2            | 0.20        |
|                             | rs28488494 | <i>AKR1C2</i> | −1.9 nmol/l                               | 212                    | 0.01                   | 6.8            | 0.31        |
|                             | rs1937863  | <i>AKR1C2</i> | −1.8 nmol/l                               | 210                    | 0.03                   | 4.6            | 0.29        |
| Model                       | Marker     | Gene          | n   | Uncorrected statistics |                        | Effect size    |             |
| Genotype (DD vs. Dd vs. dd) | rs28488494 | <i>AKR1C2</i> | 212                                       | F-test p               | F-test F               | $\eta^2$       |             |
| Model                       | Marker     | Gene          | Mean group difference in allopregnanolone | n                      | Uncorrected statistics |                | Effect size |
| Dominant (DD/Dd vs. dd)     | rs28488494 | <i>AKR1C2</i> | −2.2 nmol/l                               | 212                    | F-test p               | F-test F       | Cohen's d   |
| Model                       | Marker     | Gene          | Mean group difference in allopregnanolone | n                      | Uncorrected statistics |                | Effect size |
| Recessive (DD vs. Dd/dd)    | rs2300701  | <i>SRD5A2</i> | −2.7 nmol/l                               | 209                    | F-test p               | F-test F       | Cohen's d   |
|                             | rs806645   | <i>SRD5A2</i> | −3.5 nmol/l                               | 212                    | 0.02                   | 5.4            | 0.44        |
|                             | rs1937863  | <i>AKR1C2</i> | −5.6 nmol/l                               | 210                    | 0.02                   | 5.7            | 0.57        |
|                             |            |               |   |                        | 0.03                   | 4.8            | 0.91        |
| Model                       | Marker     | Gene          | n   | Uncorrected statistics |                        | Effect size    |             |
| Additive (dd > Dd > DD)     | rs2300701  | <i>SRD5A2</i> | 209                                       | Regression p           | Regression slope       | r <sup>2</sup> |             |
|                             | rs806645   | <i>SRD5A2</i> | 212                                       | 0.04                   | −1.3                   | 0.02           |             |
|                             | rs28488494 | <i>AKR1C2</i> | 212                                       | 0.04                   | −1.4                   | 0.02           |             |
|                             | rs1937863  | <i>AKR1C2</i> | 210                                       | 0.01                   | −1.9                   | 0.03           |             |
|                             |            |               |   | 0.04                   | −1.7                   | 0.02           |             |

such as altered GABA<sub>A</sub>-receptor sensitivity. Specifically, the expression of the  $\delta$ -subunit, which contributes to the most allopregnanolone sensitive GABA<sub>A</sub>-receptors, seems to be upregulated during pregnancy (Bhandage et al., 2015). Also, if the aforementioned polymorphisms do have an association with aldo-ketoreductase 1C2 function, its effects may also depend on the other neuroactive steroids which this enzyme can regulate (Penning et al., 2000).

Despite the support in the literature for a role of allopregnanolone in depression and anxiety, associations between genetic variation in the synthesis pathway enzymes and psychiatric symptoms in the few previously published studies have not been convincing. In a case control study on panic disorder, with SNPs spanning over the *AKR1C1-3* and *SRD5A1* genes, Quast et al. report no associations surviving correction for multiple comparisons, except for two intronic *AKR1C1* SNPs associating with a subscale of anticipatory anxiety in females only (Quast et al., 2014). Johansson et al. have found paranoid ideation in females with bipolar disorder to be linked to a four SNP *AKR1C4* haplotype (Johansson et al., 2012), while they found associations between SNPs in *SRD5A1* and *AKR1C4* and mood irritability in males but not females with bipolar disorder (Johansson et al., 2011). In addition, a genome wide association study has also identified an intronic *SRD5A1* SNP to be associated with baseline positive affect in healthy controls (Hart et al., 2012). However, beside the multifactorial origins of mood disorders, considering the multiple levels of regulation of allopregnanolone and GABA action, it is likely that any single genetic variation can only have a modest effect on psychiatric symptoms, especially during pregnancy. For example, lower plasma levels of GABA has been reported to correlate with depressive scores in pregnant women (Deligiannidis et al., 2016), and there is also preliminary evidence that pregnancy as well as psychiatric health can influence expression of GABA<sub>A</sub>-receptors (Bhandage et al., 2015).

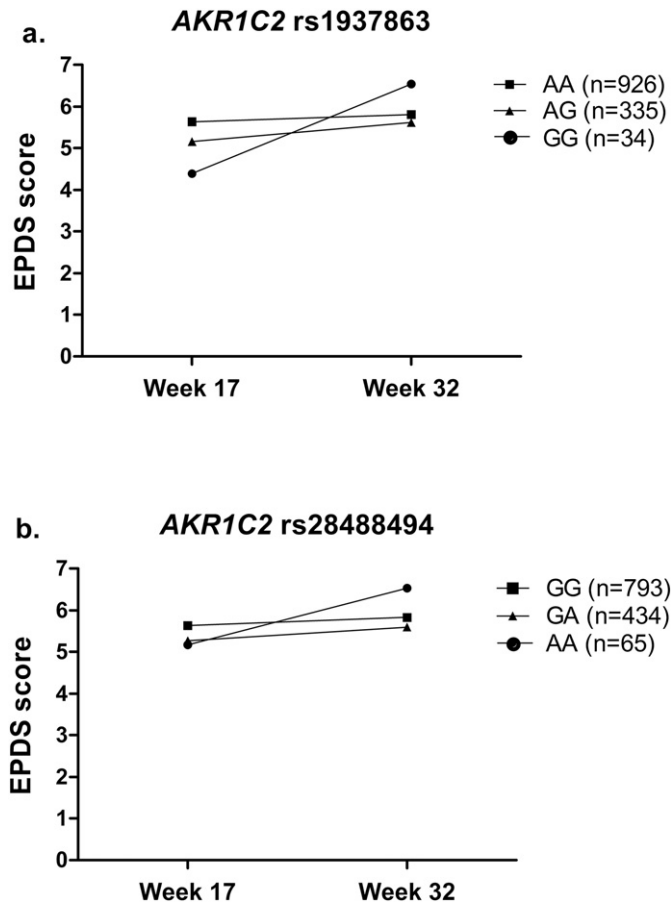
Rather than depressive symptoms, or our target genetic variations, we found that maternal BMI was the strongest predictor of plasma allopregnanolone level in mid-gestation, with higher first trimester BMI being associated with lower allopregnanolone. We can only

speculate about the reasons for this association, but a few previous findings deserve mentioning. Exogenous allopregnanolone is actively distributed to adipose tissue in rats (Zhu et al., 2004), suggesting that the lower allopregnanolone in women with higher BMI may be due to more of the steroid being sequestered in adipose tissue. It is also possible that the correlation found in our study is due to impaired placental hormone synthesis in women with high BMI (Lassance et al., 2015). However, the interpretation is complicated by the recent finding that a larger pregnancy weight gain is associated with higher plasma allopregnanolone in late pregnancy (Lundqvist et al., 2017), and the fact that overweight girls have been found to have higher allopregnanolone levels than normal weight controls (Predieri et al., 2007). Given the proposed importance of maternal allopregnanolone precursors in fetal brain development (Brunton, 2016; Cumberland et al., 2017; Vu et al., 2009), there is reason to further investigate this issue.

Methodological strengths and limitations of the present studies should be considered when interpreting the results. Among the strengths are the prospective design and the use of repeated self-report of depressive symptoms, where the first time-point was close in time to the time of blood sampling for allopregnanolone analysis. However, a longitudinal sampling of allopregnanolone levels would have enabled further analyses of relative importance of allopregnanolone in different trimesters.

Associations cannot inform about causative links, but the present genetic association analyses included a relatively large population-based sample with moderate power to be informative in relation to expected effect sizes. A dense marker coverage of the candidate genes within a pathway allowed a relatively comprehensive analysis of common variants within the genes. However, a limitation to our study is that we only analyzed SNPs from two out of the four human *AKR1C* genes. Furthermore, we have not analyzed the fetal genotype, which may influence placental steroid synthesis (Hill et al., 2011).

The profoundly changing endocrinology during pregnancy is likely to contain factors which amplify, and factors which protect from,



**Fig. 3.** Repeated measures of EPDS scores in week 17 and 32 compared between genotype groups for the *AKR1C2* SNPs rs1937863 (a) and rs28488494 (b) showing that homozygotes of the minor alleles (nominally associated with lower plasma allopregnanolone, Table 4) had a larger increase in EPDS between time-points. Interaction between rs1937863 genotype and time-point:  $F(2,1292) = 5.8, p = 0.003$ , partial  $\eta^2 = 0.009$ . Interaction between rs28488494 genotype and time-point:  $F(2,1289) = 3.3, p = 0.039$ , partial  $\eta^2 = 0.005$ .

mood disturbances. Due to its anxiolytic and antidepressant properties, allopregnanolone is a candidate for the latter category. However, we could not find evidence that plasma allopregnanolone levels during the second trimester of pregnancy were related with depressive symptoms, and conclude that measurement of central nervous system levels would be needed to elucidate whether this steroid is important for mood during pregnancy. The observation of a negative relationship between allopregnanolone and body mass is of interest in terms of central and peripheral neurophysiological interactive mechanisms regulating neuroactive steroids and obesity in pregnancy, and their possible consequences for the fetus, thus calling for further research.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.yhbeh.2017.06.008>.

### Contributors

All authors have participated in the conception of the study. E.C. has performed the statistical analyses of the genetic data. C.H. has performed the remaining statistical analyses, and drafted the first version of the manuscript. All authors have participated in the critical revision of the manuscript and approved of the final version.

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