The Effect of Common Genetic Variation in 11β-Hydroxysteroid Dehydrogenase Type 1 on Hypothalamic-Pituitary-Adrenal Axis Activity and Incident Depression

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Background: Accumulating evidence suggests that hyperactivity of the hypothalamic-pituitary-adrenal axis (HPA axis) is involved in depression. 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts inert cortisone to active cortisol and is implicated in HPA axis regulation in animal studies. The aim of our study was to identify polymorphisms in 11β-HSD1 gene (*HSD11B1*) with consistent associations with increased HPA axis activity and relate those polymorphisms to depression.

Methods: Twelve single-nucleotide polymorphisms (SNPs), including 11 tagging SNPs, were selected using the HapMap database and genotyped in 4228 participants of the population-based Rotterdam Study. The outcome measures were salivary cortisol levels after awakening, 30 min later, at 1700 h, at bedtime, and plasma levels of androstenedione (in women only). SNPs that were significantly associated with cortisol as well as androstenedione levels were also related to incident depression.

Results: rs11119328 was associated with higher cortisol saliva samples collected at bedtime as well as higher androstenedione levels (*P* value after correction for multiple testing: 0.01 and 0.04, respectively). Carriers of this polymorphism had an increased risk of an incident depression (hazard ratio 1.28, 95% confidence interval 1.03–1.59). Two other SNPs, which were in high linkage disequilibrium with rs11119328, were related to higher cortisol levels but not with androstenedione levels.

Conclusions: We identified one SNP, which was associated with increased salivary cortisol levels at nadir as well as higher androstenedione levels. Moreover, this SNP was also associated with a higher risk of an incident depression. This suggests that 11β-HSD1 is implicated in human HPA axis regulation and susceptibility to depression. **(*J Clin Endocrinol Metab* 97: E233–E237, 2012)**

Glucocorticoids are centrally regulated by the negative feedback action of the hypothalamic-pituitary-adrenal (HPA) axis: cortisol inhibits proopiomelanocortin gene transcription in the anterior pituitary and CRH gene transcription and peptide secretion in the hypothalamus (1). Local, tissue-specific cortisol concentrations are fine-tuned by two enzymes, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and 11β-hydroxysteroid dehydrogenase type 2. *In vivo*, 11β-HSD1 converts cortisone to cortisol, whereas 11β-

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Abbreviations: $Cort_{bed}$, Cortisol saliva samples collected at bedtime; HPA, hypothalamic-pituitary-adrenal; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; SNP, single-nucleotide polymorphism.

hydroxysteroid dehydrogenase type 2 converts cortisol to cortisone (2).

Animal studies strongly suggest that 11β-HSD1 also plays a role in central HPA axis regulation. In rodents, 11β-HSD1 expression is observed in the anterior pituitary, the paraventricular nucleus (PVN), and the hippocampus (2–4). Furthermore, 11β-HSD1-deficient mice showed elevated basal corticosterone and ACTH levels and exaggerated ACTH and corticosterone responses to restraint stress, suggesting diminished glucocorticoid feedback (5).

In the present study, we evaluated the associations of common genetic variation in *HSD11B1* with HPA axis activity using a tagging single-nucleotide polymorphism (SNP) approach. As markers for HPA axis activity, we used salivary cortisol levels and in women also adrenal androgens. Our aim was to identify SNP that show consistent associations with increased HPA axis activity and relate those polymorphisms to incident depression. To test these hypotheses, we used data from the Rotterdam Study, a population-based cohort study in an elderly population.

Materials and Methods

Study population

The present study was embedded in the Rotterdam Study, an ongoing population-based cohort study of persons aged 55 yr and older (6). So far, four study surveys have taken place in which participants underwent different measurements. A time line of these study surveys is given in Supplemental Fig. 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org.

Plasma androstenedione measurement

Analyses with androstenedione were performed in women only because in postmenopausal women the adrenals are the main source of androgen production, and its release is under the control of the HPA axis. In a limited number of women who participated in the baseline examinations (1990–1993, n = 883), plasma levels of androstenedione were determined as described previously (7). Androstenedione is stable in frozen plasma (8). Women using exogenous hormones (n = 39) were excluded. This resulted in a study population of 844 women.

Salivary cortisol

During the fourth study, survey (2002–2004) participants collected saliva samples at four different time points: directly after awakening, 30 min later, at 1700 h, and at bedtime ($Cort_{bed}$). Details on saliva collection and cortisol measurements have been described previously (9). For each time point, cortisol values that were above the 98th percentile were excluded. This conservative cutoff was chosen due to the high number of outliers, in line with earlier studies (9). Moreover, the 98th percentile of late-night salivary cortisol levels (13.4 nmol/liter) corresponded approximately to the cutoff value used in the diagnosis of Cushing's syndrome. Persons using systemic glucocorticoids (n = 49) were excluded. This resulted in a study population of 2190 participants.

Incident depression

Depression screening followed a multistep protocol and has been described in detail previously (10). In short, screening for depression had started during the second study round (1993–1995) using standardized questionnaires. Information on the occurrence of depressions during follow-up was obtained from psychiatric examinations during the examination rounds, self-reported histories of depression, medical records, and registration of antidepressant use via an automatic link with the pharmacies that serves the study area. An event was considered a depressive syndrome if one of the following conditions was met: a *Diagnostic and Statistical Manual of Mental Disorders*-defined major or minor depression diagnosed by a psychiatrist or another mental health professional, depressions recorded by a general practitioner (GP) or other physician, and a self-reported depression for which the participant consulted a GP or a mental health professional. We defined the date of onset as the day of the first report of symptoms or the first prescription date of an antidepressant drug, whichever came first.

At baseline (second study round), we excluded 549 persons with depressive symptoms, 105 with dementia, nine with bipolar disorder, and two lost to follow-up directly after screening. This resulted in a study population of 4228 persons.

DNA analysis

HSD11B1 tagging SNPs were selected using the HapMap database (public release no. 20). SNPs were selected to tag common variation (allele frequency cutoff 5%) 5 kb upstream and 1 kb downstream of *HSD11B1*. This resulted in the selection of 11 tagging SNPs. Because *HSD11B1* 83,557insA (rs45487298), which is in full linkage disequilibrium (LD) with a T>G substitution at position 83,597 (rs12086634), has been extensively studied previously, we also included this polymorphism in our analyses.

The appropriate Assay-by-Design mixes were obtained from Applied Biosystems (Foster City, CA). See Supplemental Table 1 for primer sequences. Plates were analyzed using the Applied Biosystems 7900HT sequence detection system and sequence detection system version 2.0 software (Applied Biosystems). To confirm the accuracy of genotyping results, 5% of the samples were regenotyped. Percentage of concordance was higher than 99% for all SNPs.

Statistical analyses

Hardy-Weinberg equilibria were calculated using χ^2 analyses. Haploview (release 4.1) was used to determine the LD blocks and the r^2 values. All hormone levels were log transformed to normalize distributions.

First, linear regression models were used to study the effects of *HSD11B1* SNPs on hormonal measures. *P* values were corrected for multiple testing using a permutation procedure (10,000 permutations) available in the R package GenABEL version 1.6–4 (11). Next, we studied the effects *HSD11B1* SNPs on the incidence of depressive disorder using proportional hazard analyses. To minimize multiple testing, only SNP that were associated with higher salivary cortisol as well as higher androstenedione levels were included in these analyses. Subjects were analyzed as carriers (one or two copies of minor allele) *vs.* non-

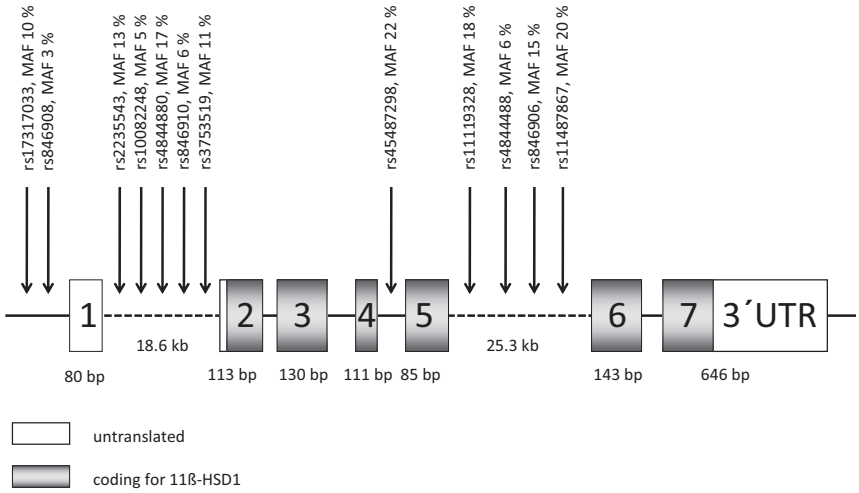

FIG. 1. Schematic overview of *HSD11B1*, selected tagging SNPs, and minor allele frequencies (MAF).

carriers. All analyses were adjusted for age and sex. Statistical analyses were performed using SPSS for Windows, release 15.0 (SPSS Inc., Chicago, IL). *Post hoc* analyses were performed to study the effect of *HSD11B1* SNPs on hormonal levels in persons without a history of depression.

Results

Figure 1 shows a schematic overview of *HSD11B1* and the selected SNPs. Additional SNP information is provided in Supplemental Table 2 and Supplemental Fig. 2. Population characteristics are given in Supplemental Table 3.

Of the 12 SNPs, only one SNP, rs11119328, showed consistent associations with higher levels of salivary cortisol at bedtime as well as androstenedione in women (Table 1). These associations remained statistically significant after correction for multiple testing. Two other SNPs, rs11487867 and rs45487298, which are in high LD with rs11119328 (Supplemental Fig. 2), were significantly associated with higher salivary cortisol at bedtime but not with androstenedione (Table 1). None of the analyses with other *HSD11B1* SNPs and hormonal measures reached statistical significance.

Post hoc analyses showed that rs11119328 was also associated with higher $Cort_{bed}$ levels in persons without a history of depression at the fourth study survey (n = 1121, $B_{Cort(bed)}$ = 0.056 $Log_{nmol/liter}$ [95% confidence interval (CI) 0.015–0.097 $Log_{nmol/liter}$), $P = 0.007$]. A similar trend was not observed for androstenedione after exclusion of persons with a history of depression [n = 251, B = 0.006 $Log_{nmol/liter}$ (95% CI −0.027–0.066 $Log_{nmol/liter}$), $P = 0.414$].

We used Cox proportional hazard analyses to study the effect of *HSD11B1* SNPs on incidence of depression. For these analyses only one SNP, rs11119328, which was associated with higher $Cort_{bed}$ as well as higher androstenedione levels, was selected. Carriers of rs11119328 had a significantly higher chance of having an incident depressive syndrome: hazard ratio 1.28, 95% CI 1.03–1.59. Follow-up time consisted of 35413 person-years, with a mean follow-up time of 8.4 yr per subject. A total of 351 subjects were diagnosed with an incident depressive syndrome.

Discussion

The present study shows that a common genetic variant in *HSD11B1*, rs11119328, is associated with higher late-night salivary cortisol levels and in postmenopausal

TABLE 1. Associations of *HSD11B1* SNPs and adrenal hormone levels

	rs11119328			rs11487867			rs45487298		
	β (95% CI)[a]	*P* value[a]	$P_{corrected}$ value[b]	β (95% CI)[a]	*P* value[a]	$P_{corrected}$ value[b]	β (95% CI)[a]	*P* value[a]	$P_{corrected}$ value[b]
Cortisol measures (n = 2190)[c]									
$Cort_{aw}$, $Log_{nmol/liter}$	0.004 (−0.021 to 0.028)	0.78	1	0.006 (−0.018 to 0.030)	0.63	0.99	0.010 (−0.012 to 0.033)	0.38	0.98
$Cort_{aw\ +\ 30}$, $Log_{nmol/liter}$	0.028 (0.005 to 0.051)	0.02	0.16	0.017 (−0.006 to 0.039)	0.15	0.73	0.020 (−0.001 to 0.042)	0.07	0.44
$Cort_{1700\ h}$, $Log_{nmol/liter}$	0.027 (0.002 to 0.053)	0.03	0.27	0.029 (0.005 to 0.054)	0.02	0.18	0.030 (0.007 to 0.054)	0.01	0.10
$Cort_{bed}$, $Log_{nmol/liter}$	0.049 (0.019 to 0.080)	0.001	0.01	0.044 (0.014 to 0.073)	0.004	0.04	0.044 (0.016 to 0.072)	0.002	0.02
Adrenal androgen (n = 844)[c]									
Androstenedione, $Log_{nmol/liter}$	0.039 (0.012 to 0.067)	0.005	0.04	0.016 (−0.010 to 0.042)	0.23	0.87	0.024 (−0.001 to 0.050)	0.06	0.41

$Cort_{aw}$, Cortisol saliva samples collected directly after awakening; $Cort_{aw+30}$, cortisol saliva samples collected 30 min later; $Cort_{1700\ h}$, cortisol saliva samples collected at 1700 h.

[a] Values represent β -coefficients, 95% CI, and *P* values adjusted for age and sex (cortisol measures) or age (androstenedione).

[b] *P* value additionally corrected for multiple testing by permutation analyses.

[c] Numbers differ slightly between analyses due to differences in genotype success rates between SNPs and differences in number of available saliva samples at different time points. Please note that values represent log-transformed hormonal levels. Mean untransformed and log-transformed hormonal values are given in Supplemental Table 3.

women with higher androstenedione levels. This polymorphism was also related to an increased susceptibility to depression. Two other *HSD11B1* SNPs, which were in high LD with rs11119328, were related to higher cortisol levels but not with androstenedione levels.

Several observations support the concept that 11β-HSD1 plays an important role in HPA axis regulation. In rodents, 11β-HSD1 is highly expressed at the central feedback sites of the HPA axis (2–4). Harris et *al.* (5) showed that 129/MF1 mice lacking 11β-HSD1 activity show elevated basal corticosterone and ACTH levels and exaggerated ACTH and corticosterone responses to restraint stress, suggesting diminished glucocorticoid feedback. In our study we found an association between rs11119328 and increased measures of HPA axis activity at two different points in time: higher cortisol levels at nadir (measured 1990–1993) as well as higher androstenedione levels (measured 2002–2004), indicating a higher set point of the HPA axis. One possible explanation is that this SNP is associated with lower expression of 11β-HSD1 at the central feedback sites of the HPA axis or peripherally resulting in diminished negative feedback.

Diminished HPA axis negative feedback is thought to be a key element in depressive disorder neurobiology. So far, it is not yet clear whether these changes in HPA axis function are consequences of central neurotransmitter changes occurring during depression. Or, alternatively, that increased HPA axis activity plays a causal role in the etiology of depression. Our study was not designed to infer causality between HPA axis activity and depression. Therefore, the observed association between rs11119328 and depression might be the result of increased HPA axis activity or, alternatively, the observed increased hormonal levels might the result of a previous depression. *Post hoc* analyses showed that rs11119328 was also associated with higher $Cort_{bed}$ levels in persons without a history of depression, suggesting that this association is not the result of a previous depressive episode. However, a similar trend was not observed for androstenedione.

Some other methodological issues of our study need to be discussed. First, studies using saliva sampling to determine the diurnal cortisol pattern rely heavily upon participant compliance. Kudielka *et al.* (12) showed in a study with electronic monitoring devices that a significant number of participants in an ambulatory setting did not obtain saliva samples reliably. The most important effect of compliance on the cortisol measurement was seen for the cortisol awakening response. Compliant subjects showed a robust increase in cortisol values 30 min after awakening, whereas participants who failed to obtain this sample at the correct timing only showed minimal changes from baseline. The influence of variation in sampling time is much smaller for evening cortisol levels because these levels are more stable. Noncompliance to the study protocol has probably also influenced the salivary cortisol concentrations in our study. This might explain the negative findings for the relationship between *HSD11B1* SNPs and morning cortisol levels.

Second, genetic association studies are prone to both type 1 and type 2 errors. Large numbers of subjects are needed because effect sizes for common variants are typically modest (13, 14). Our study was designed to evaluate the effects of *HSD11B1* SNPs on salivary cortisol measures (n = 2190) and depression (n = 4228). Possibly our study was underpowered to detect significant effects of the two SNPs that are in strong LD with rs11119328 (rs11487867 and rs45487298) on androstenedione levels (n = 844). Also, multiple testing is an issue, and we adjusted for this by using permutation analyses. However, replication studies are the golden standard and future studies are needed to confirm our results.

In summary, we showed that one tagging SNP in *HSD11B1*, rs11119328, is associated with increased late-night cortisol levels and in postmenopausal women with higher androstenedione levels. Carriers of this polymorphism are also at increased risk of developing a depressive episode. These findings suggest that 11β-HSD1 is implicated in human HPA axis regulation and depressive disorder neurobiology.

Acknowledgments

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