

***PCLO* rs2522833 impacts HPA system activity in healthy young adults**

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Recent genetic studies showed evidence for a role of the single-nucleotide polymorphism rs2522833 within the *PCLO* gene in the etiology of major depression, and rs2522833 has been shown to modulate hypothalamic pituitary adrenal (HPA) axis activity during antidepressant treatment. Monoaminergic modulation of the HPA system may be one possible pathomechanism by which *PCLO* exerts its effect on depression. In the present study, we investigated the effect of rs2522833 on the cortisol awakening response (CAR) in healthy young adults. A total of 66 healthy volunteers from the community (36 men and 30 women) aged 18–25 years without individual or family history of affective disorders and schizophrenia collected saliva cortisol samples at 0, 30, 45 and 60 min after awakening on two consecutive working days. We identified a blunted CAR (AUCinc) in rs2522833 risk-allele (C) carriers, possibly indicating exhausted regulatory mechanisms underlying the HPA system. We also identified higher neuroticism scores in rs2522833 risk-allele carriers but no phenotypic correlation between the CAR (AUCinc) and neuroticism. These findings suggest that the rs2522833 risk variant might increase vulnerability to depression both by physiological and behavioral pathways, which appear, however, not to be substantially overlapped. Replication with larger samples is warranted. *Translational Psychiatry* (2011) 1, e10; doi:10.1038/tp.2011.11; published online 31 May 2011

Introduction

A recent genome-wide association study¹ showed evidence for a role of the single-nucleotide polymorphism rs2522833 within the piccolo (*PCLO*) gene in the etiology of major depression, which was supported by a subsequent meta-analysis² and joint reanalysis of correlated multiple single-nucleotide polymorphisms.³ Another region in *PCLO* has been associated with bipolar disorder.⁴ Furthermore, rs2522833 modulated hypothalamic pituitary adrenal (HPA) axis activity during antidepressant treatment.⁵ In this report, we show that rs2522833 impacts HPA axis regulation in healthy young adults.

The protein product of the *PCLO* gene is relevant for monoaminergic neurotransmission in the brain, making it a plausible candidate gene for affective disorders. Although the original genome-wide association study¹ and the subsequent meta-analysis² replicated positive findings only in population-based samples, rs2522833 influence on HPA system response to antidepressants has been observed in depressed patients, referred as inpatients.⁵ Here, C-allele carriers showed larger HPA dysregulation than AA-carriers. This study suggests monoaminergic modulation of the HPA system as one possible pathomechanism by which *PCLO* exerts its effect on depression.

In the present study, we sought to investigate the effect of rs2522833 on HPA system activity in healthy young adults. Investigating healthy samples allows to measure effects of

common gene variants on physiological and behavioral phenotypes, which are not yet confounded with but possibly increase vulnerability to depression. As a physiological marker, we investigated the cortisol awakening response (CAR), denoting the rise in cortisol levels during the first hour after awakening. The CAR reflects the sensitivity of the HPA axis in response to a naturally occurring stressor (awakening) and has gained growing attention because of its distinct association with stress and psychopathology,⁶ and its higher genetic liability than daytime cortisol levels.⁷ In depression, both increased and blunted CARs have been identified,⁷ possibly because of different subgroups and/or severity of illness. A recent meta-analysis that also included community studies concluded that the CAR is negatively correlated with depression, especially its dynamic component.⁷ A blunted morning rise in cortisol levels has also been identified in healthy subjects with cognitive vulnerability to depression.⁸ Therefore, we expected particularly the dynamic aspect of the CAR to be lower in C-allele carriers.

In parallel to physiological phenotypes, personality traits may underlie the association between rs2522833 and vulnerability to depression. Particularly neuroticism has been shown to have a clear genetic overlap with major depression and to predict the subsequent onset of the disorder.⁹ Furthermore, as neuroticism reflects a tendency to cope poorly with stress,⁹ HPA axis (dys)function may be involved in this association. Therefore, we also aimed to investigate the association

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Keywords: *PCLO* gene; HPA axis; cortisol awakening response; neuroticism

Received 23 March 2011; accepted 15 April 2011

between rs2522833 and neuroticism, as well as a possible phenotypic relationship between the CAR and neuroticism.

Subjects and methods

We recruited 66 healthy volunteers aged 18–25 years (36 men and 30 women) from the community without individual or family history of affective disorders and schizophrenia. On two consecutive working days, participants collected four saliva cortisol samples per day during the first hour after awakening (at awakening, +30 min, +45 min and +60 min). Participants were instructed not to brush their teeth or eat, drink and smoke during sampling periods, and to record exact sampling times and adherence to the protocol.

Saliva samples were analyzed using a time-resolved fluorescence immunoassay. Intra- and interassay variability were <10%. Cortisol samples for each time point were averaged across days. If subjects did not adhere to the protocol for a probe, these values were excluded from analyses. For the CAR, we calculated both the area under the curve with respect to increase (AUC_{inc}) and with respect to the ground (AUC_g). The AUC_{inc} denotes the dynamic fluctuation of the system from the awakening baseline, whereas AUC_g is an estimate of total cortisol secretion during the first hour.¹⁰ Analyses of covariance involving genotype (AA- versus C-allele carriers) as independent factor and CAR components as dependent variables were controlled for depressive symptom levels, sex, age and habitual smoking.

Depressive symptoms were measured with the Beck Depression Inventory, second revision,¹¹ and neuroticism was measured with the Neo-Five-Factor Inventory subscale neuroticism.¹² The analysis of covariance involving neuroticism as dependent variable was controlled for depressive symptoms.

Rs2522833 was genotyped using a TaqMan 5' nuclease assay. A duplication of 15% of the sample revealed reproducibility of 100%. Allele frequencies (AA: 30.3%, AC: 56.1% and CC: 13.6%) were in Hardy–Weinberg equilibrium ($P > 0.05$). Owing to small numbers of CC homozygotes, we pooled subjects with AC and CC genotypes (risk-allele carriers, $n = 46$).

Results and discussion

Compared with rs2522833 AA-carriers, C-allele carriers showed significantly lower CAR_AUC_{inc} than AA-carriers (282.0 ± 319.1 versus 467.0 ± 269.1 , $F(1,61) = 4.35$, $P = 0.042$, see Figure 1), but similar CAR_AUC_g (1074.3 ± 305.2 versus 1115.7 ± 368.2 , $F(1,61) = 0.07$, not significant). Furthermore, C-carriers scored higher on neuroticism than AA-carriers (18.6 ± 6.7 versus 15.5 ± 6.7 , $F(1,63) = 4.17$, $P = 0.045$). However, by adjusting for age, sex and habitual smoking, neuroticism was not correlated with CAR_AUC_{inc} ($r_{partial} = -0.116$, not significant), and its inclusion as a further covariate in a respective analysis of covariance model did not affect the significant association between rs2522833 genotype and CAR_AUC_{inc} ($F(1,60) = 4.40$, $P = 0.041$).

Although these results clearly need replication in larger samples, they point to a possible influence of rs2522833 on

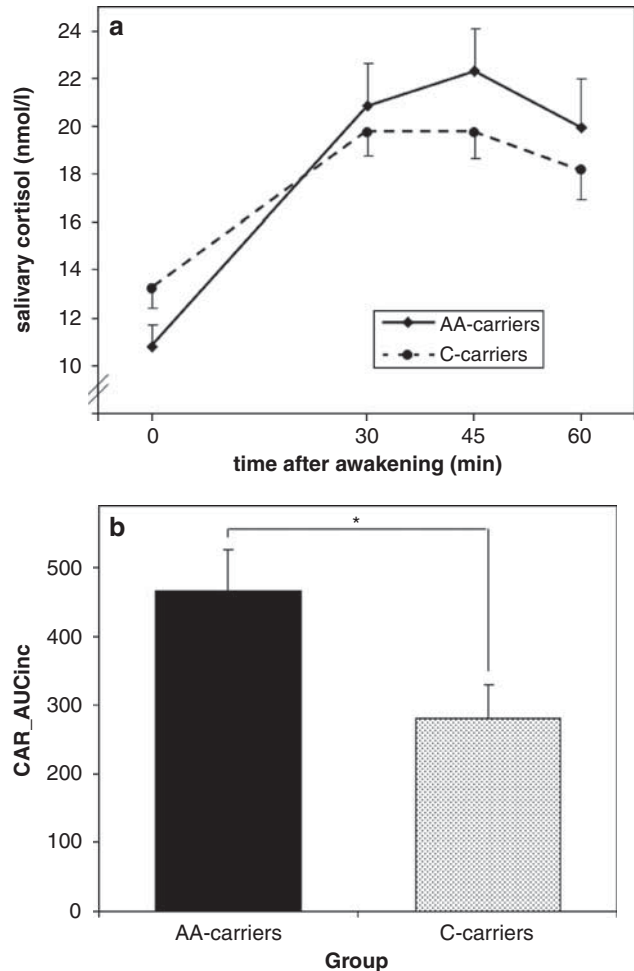


Figure 1 The cortisol awakening response (CAR) for rs2522833 AA- and C-allele carriers. (a) Course of cortisol during the first hour after awakening. (b) The CAR area under the curve with respect to increase (AUC_{inc}). In both graphs, error bars represent standard errors of the mean. * $P < 0.05$.

both HPA activation and neuroticism. These associations were not due to genotype group differences in subclinical symptoms, which were controlled in the respective models. Both dysregulation of the CAR and neuroticism have been identified as vulnerability factors for depression.^{6–10} As hypothesized, rs2522833 was associated particularly with the dynamic component of the CAR,⁷ and the blunted CAR increase in risk-allele carriers might indicate exhausted regulatory mechanisms underlying the HPA system. Furthermore, we identified an association between rs2522833 genotype and neuroticism, but no phenotypic correlation between CAR_AUC_{inc} and neuroticism. Therefore, our results suggest that the rs2522833 risk variant might increase vulnerability to depression both by physiological and by behavioral pathways, which appear, however, not to be substantially overlapped. Alternatively, unknown factors may have masked the relationship between CAR_AUC_{inc} and neuroticism. Finally, it is not yet known whether rs2522833 itself or a variant in disequilibrium with it is causally linked to physiological and behavioral phenotypes.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements. This project was supported by the German Research Foundation (DFG KU1464/4-1 and SFB636 D4, Z4).

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