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Short Communication

Association analyses of depression and genes in the hypothalamus–pituitary–adrenal axis

Buttenschøn HN, Krogh J, Nielsen MN, Kaerlev L, Nordentoft M, Mors, O. Association analyses of depression and genes in the hypothalamus–pituitary–adrenal axis.

Objective: Dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis has been reported in depression. The aim was to investigate the potential association between depression and seven genes regulating or interfering with the HPA axis, including the gene encoding angiotensin converting enzyme (*ACE*).
Methods: In total, 78 single nucleotide polymorphisms (SNPs) and one insertion/deletion polymorphism were genotyped. The study included 408 individuals with depression and 289 controls. In a subset of cases, the interaction between genetic variants and stressful life events (SLEs) was investigated.
Results: After quality control, 68 genetic variants were left for analyses. Four of nine variants within *ACE* were nominally associated with depression and a gene-wise association was likewise observed. However, none of the SNPs located within *AVP*, *CRH*, *CRHR1*, *CRHR2*, *FKBP5* or *NC3C1* were associated with depression. One nominally significant interaction, most likely due to chance, was identified.
Conclusion: The results indicate that *ACE* could be a potential candidate gene for depression.

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Significant outcomes

- In this comprehensive candidate gene study investigating 79 genetic variants located in seven genes known to interfere with or to be involved in the regulation of the hypothalamus–pituitary–adrenal axis, we identified the following.
- Four genetic variants in *ACE* were nominally significantly associated with depression.
- Subsequent analyses showed a gene-wise association between *ACE* and depression.
- No associations between depression and genetic variants in *AVP*, *CRH*, *CRHR1*, *CRHR2*, *FKBP5* or *NC3C1* and were observed.

Limitations

- The relatively small sample size – larger studies are warranted.
- It was only possible to perform the gene–environment interaction analyses in a subset of cases.
- The limited data decrease the statistical power for the gene–environment interaction analyses.

Introduction

Depression is a genetically complex trait with a heritability of 31–42% (1). Thus, depression results from a complex interplay of genes and environmental factors. Interactions between genes and environmental factors (including stressful life events (SLEs)) are assumed to modify the risk for depression (2).

Dysregulation of the stress responsive hypothalamic–pituitary–adrenal (HPA) axis is one of the best documented biological findings in depression. The underlying mechanisms are however unclear (3). The HPA axis constitutes a complex set of interactions among the hypothalamus, the pituitary gland, and the adrenal gland. The corticotrophin releasing hormone (CRH) and arginin vasopressin (AVP) are secreted from the hypothalamus and stimulate the release of corticotrophin (adrenocorticotropin hormone, ACTH) in the pituitary gland, which subsequently stimulates the synthesis of glucocorticoids (mainly cortisol in humans) from the adrenal cortex. The binding of glucocorticoids to their receptors suppresses the production of CRH and ACTH in a negative feedback cycle (3).

The angiotensin converting enzyme (ACE) is an essential enzyme in the renin–angiotensin system which converts inactive angiotensin I into active angiotensin II, leading to a constriction of the blood vessels and increased blood pressure. Several lines of evidence also indicate an interaction between the renin–angiotensin system (including ACE) and the HPA axis (4,5). ACE-inhibitors have likewise shown anti-depressant properties (6).

The main aim of the present study was to investigate the potential association between depression and seven genes regulating or influencing the HPA axis by analysing 79 genetic variants. A secondary aim of the study was to investigate the interaction between genetic variants and SLEs for association with depression.

Methods

Populations

We examined genetic markers for association with depression using four Danish cohorts comprising 408 individuals with depression and 289 ethnically matched controls (Table 1). All cases fulfilled the ICD-10 diagnostic criteria for depression. Diagnoses were obtained using a semi-structured diagnostic interview (Schedules for Clinical Assessment in Neuropsychiatry (SCAN version 2.1)) (7) except for the Denmark IV cohort where the Major Depression Inventory was used. In the Denmark II and IV cohorts, the measures of depression severity were assessed by different rating scales, including the Montgomery–Åsberg Depression Rating Scale (MADRS) (8).

Table 1. Sample characteristics

Cohorts	Total number	Male (%)	Female (%)	References
Patients	408	99 (24%)	309 (76%)	
Denmark I	162	27 (17%)	135 (83%)	(21,22)
Denmark II	80	24 (30%)	56 (70%)	(23)
Denmark III	62	20 (32%)	42 (68%)	(24)
Denmark IV	104	28 (27%)	76 (73%)	(25)
Controls	289	58 (20%)	231 (80%)	(21,22)

In the Denmark I cohort comprising 162 depressed individuals, information on SLEs was available. A Danish translation of a brief version of the List of Threatening Experiences Questionnaire (LTE-Q) (9,10) was used to record nine SLEs rated on a four-point severity scale. In this analysis, only moderate and severe events were counted. Compared with the original LTE-Q consisting of 12 events, we excluded two questions in relation to unemployment since all participants were employees at major public sector workplaces in a Danish county. In addition, two questions regarding separation due to marital difficulties or break of a steady relationship were combined into a single item.

The control sample consisted of screened individuals without diagnoses of affective or anxiety disorders. Information on SLEs was also available for the control cohort.

All cases and controls were Caucasian of origin and gave written informed consent. The study has been approved by the Danish Data Protection Agency and the ethics committees in Denmark.

Genotyping

Single nucleotide polymorphisms (SNPs) selected for genotyping were identified from HapMap data (http://www.hapmap.org/) using the tagger selection algorithm in Haploview (11).

Briefly, 78 SNPs located within seven genes [13 SNPs within *ACE*, three SNPs within *AVP*,

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three SNPs within *CRH*, 15 SNPs within the gene encoding corticotropin releasing hormone receptor 1 (*CRHR1*), 14 SNPs within the gene encoding corticotropin releasing hormone receptor 2 (*CRHR2*), eight SNPs within the gene encoding FK506 binding protein 5 (*FKBP5*) and 22 SNPs within the gene encoding the Nuclear Receptor Subfamily 3, Group C, Member 1 (*NR3C1*)] were genotyped using the Sequenom MassARRAY platform and analysed using the MassARRAY Typer software (version 4.0). All cluster plots were manually inspected by two experienced investigators. The analysed genes were selected based on their regulation of the HPA axis and previous genetic association studies in depression.

In addition, one insertion/deletion polymorphism within the *ACE* gene (*ACE* I/D) was genotyped and analysed using the ABI 3130 Prism Genetic Analyzer and the Genemapper software version 4.0 (Applied Biosystems, Fostercity, CA, USA). The forward primer was modified using a FAM fluorescein. Polymerase chain reaction products were directly used for genotyping to reveal the 'inserted' (478 bp) or 'deleted' (191 bp) allele. Two experienced investigators individually inspected the genotypes.

Additional method conditions and primer sequences are available on request.

Statistical analyses

The software PLINK (12) was used to perform quality control and association analyses. After quality control, 67 SNPs (Supplementary Table 1) with well-defined cluster plots, in Hardy-Weinberg equilibrium among control individuals ($p > 0.01$), minor allele frequencies above 0.005 and a call rate higher than 94% were left for analyses. To increase the genotypic information, additional genotypes were imputed using the software MACH 1.0 (13) and the 1000 Genomes (CEU population) as reference haplotypes. To overcome typical strand issues, A/T and G/C SNPs were excluded before imputation. Only imputed SNPs with a squared correlation between imputed and true genotypes above 0.3 and a quality score above 0.9 were kept for further analyses. COMBASSOC was used to evaluate the strength of evidence for association of genes (14). Using this approach, the evidence of the association is first combined and second, the strength of gene-wise association is assessed using permutations.

To evaluate the potential effect of the *ACE* I/D polymorphism on the MADRS depression severity score in the Denmark II and IV cohorts, linear regression analyses were performed. The association between depression and genetic variants, SLEs and their interactions was investigated in the Denmark I cohort using multiple logistic regression analyses. All regression analyses were performed using Stata13.

Results

None of the successfully analysed SNPs within *AVP*, *CRH*, *CRHR1*, *CRHR2*, *FKBP5* or *NC3C1* were associated with depression (not shown). In contrast, three of eight successfully genotyped SNPs within *ACE* were nominally associated with depression (Table 2), and we likewise observed a significant gene-wise association between *ACE* and depression ($p = 0.00057$, $p_{empirical} = 0.026$).

The most associated SNP (rs4309) is a synonymous SNP. Rs4311 and rs4329 were also associated with depression but located within introns, 840 and 3535 bp distal to rs4309, respectively. The pairwise linkage disequilibrium (LD) between the nominally associated SNPs in the present study is relatively high ($r^2 > 0.56$).

The *ACE* I/D polymorphism was likewise nominally associated with depression (Table 2).

As the occurrence of depression is higher in females than in males, we also performed gender specific analyses. In the female subset comprising 309 cases and 231 controls, only minor changes, compared with the result presented in Table 2, were observed (not shown). However, in the small subset of males (99 cases and 58 controls), no association was observed (not shown). Imputation was performed on seven SNPs within *ACE* and allowed us to obtain genotypic information from additional 47 SNPs of which 24 were nominally associated with depression (not shown).The allele counts (and *p*-values) of all 24 SNPs were identical to that of rs4329. Thus, the gender specific analyses and the imputation analyses did not add additional information to the results.

Using linear and logistic regression analyses, we assessed the potential effect of the *ACE* I/D polymorphism on depression severity in addition to the potential association between SLEs and depression and the potential interaction between SLEs and genetic variants. No association was, however, observed between the *ACE* I/D polymorphism and depression severity in the Denmark II and IV cohorts (not shown). In contrast, SLEs was significantly associated with depression ($p = 0.001$). The results of the interaction analyses revealed no significant interactions between the nominally associated SNPs in *ACE* and SLEs. However, one nominally significant interaction between a SNP located downstream of the *CRH* gene (rs6982394) and SLEs ($p = 0.012$) in the Denmark I cohort was observed. Further

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Table 2. Trend test for association between genetic markers in *ACE* and depression

Marker	BP*	SNP	Function	Genotypes (cases)	Genotypes (controls)	MAF (cases)	MAF (controls)	CHISQ	*p*-value
RS8076157	61 543 861	**T**/C	5′-upstream	30/162/211	21/102/157	0.28	0.26	0.56	0.46
RS4295	61 556 298	**G**/C	Intron	55/178/165	44/141/97	0.36	0.41	2.7	0.099
RS3730025	61 557 773	**G**/A	Missense	0/10/393	0/12/271	0.012	0.021	1.6	0.20
RS4309	61 559 923	**T**/C	Coding-synonymous	87/137/111	46/113/108	0.46	0.38	6.8	0.0092
RS4311	61 560 763	**T**/C	Intron	78/190/123	73/119/71	0.44	0.50	4.5	0.033
RS4329	61 563 458	**G**/A	Intron	105/200/99	59/134/91	0.51	0.44	5.3	0.022
RS4461142	61 578 048	**T**/C	3′-downstream	88/193/121	76/132/71	0.46	0.51	3.2	0.075
RS4316818	61 584 627	**T**/C	3′-downstrean	75/210/119	48/149/87	0.45	0.43	0.29	0.59
ACE I/D		**I**/D	Intron	103/200/102	61/134/92	0.50	0.45	4.0	0.046

BP, base pair; SNP, single nucleotide polymorphism; MAF, minor allele frequency; CHISQ, chi-squared test statistics.
* The position of the analysed SNPs according to dbSNP NCBI build 138. Minor alleles are shown in bold.

interpretation of this interaction shows that, significantly fewer heterozygous cases who have experienced SLEs (8%) compared with heterozygous cases who have not experienced SLEs (23%) were observed. No difference was observed in the control group. However, we observed a tendency towards more heterozygous individuals with SLEs (15%) compared with those without SLEs (11%). The frequency of the minor allele was 0.07, and no individuals homozygous for the minor allele were included in the logistic regression analyses.

Discussion

In summary, the study investigated the association between depression and genetic variants in seven genes involved in the regulation of the HPA axis. Interestingly, we observed association between four genetic variants located within the *ACE* gene and depression, although none of the variants remained significantly associated after correction for multiple testing. No association was observed between depression and variants in the remaining genes.

ACE has been recognised as a candidate gene for cardiovascular disease in addition to depression and was thus included in the present study. The association between common polymorphisms within *ACE* and depression has been frequently investigated, and the most widely studied polymorphism has been the *ACE* I/D. A meta-analysis from 2012 comprising 15 studies and more than 10 000 individuals showed 18% increased risk of depression in individuals homozygous for the deleted allele compared with individuals heterozygous or homozygous for the inserted allele (15). In subgroup analyses by ethnicity, association was observed in the Caucasian population, but not in the Asian population (15). In the present study, we observed a nominal association between the *ACE* I/D polymorphism and depression. However, in contrast to the results from most other studies (15), the deleted allele was more common among the controls compared with the cases. In agreement with a previous study (16), we observed no association between the *ACE* I/D polymorphism and depression severity. Although not directly comparable, this is however in contrast to a recent study by Hui et al. reporting a significantly lower depressive symptom score in individuals with schizophrenia being homozygous for the deleted allele compared with individuals homozygous for the inserted allele (17).

A study by Baghai et al. (18) investigated the association between several SNPs within *ACE* and depression in two independent case/control cohorts comprising 843 patients with depression and 1479 controls. One SNP (rs4291) located within the promotor region of the gene was significantly associated in both samples. The LD between rs4291 and rs4311 (nominally associated in our study) is relatively high ($r^2 > 0.6$, D' > 0.9). Rs4291 was imputed in our study, however, excluded from further analyses since the quality score was below 0.7. Interestingly, rs4291 was shown to influence ACE activity and HPA axis hyperactivity (18). A recent study using multi-adjusted logistic regression analyses observed significant association between seven SNPs within *ACE* and late-life depression (19). Two of the associated SNPs from that study (rs4295 and rs4311) were also included in our study. Similar to the study by Ancelin et al. (19), the GG and TT genotypes of rs4295 and rs4311, respectively, were more common among the controls compared with cases, and rs4311 was likewise nominally associated in our study.

We also systematically selected and investigated genetic variation within the genes encoding the three hormones: CRH, AVP and ACTH for association with depression. Furthermore, genetic variation within the receptors for CRH (*CRHR1* and *CRHR2*)

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and the glucocorticoid receptor (*NR3C1*) was investigated. Since the regulation of NR3C1 is dependent on the co-chaperone FKBP5, we also investigated genetic variation in this gene for association with depression.

Several candidate gene studies have previously investigated genes regulating the HPA axis for association with depression. Most of these studies have focussed on a limited number of genetic variants located within *FKBP5*, *CRHR1* and *NR3C1* and the results have been conflicting. In contrast to these studies, we systematically selected and analysed tagSNPs to retain as much as possible of the genetic variation of a gene using a minimal number of SNPs. We successfully analysed two SNPs in *AVP*, three in *CRH*, 13 in *CRHR1*, 14 in *CRHR2*, seven in *FKBP5* and 20 SNPs in *NC3C1* but found no evidence for association with depression.

Several lines of evidence have suggested that gene–environment interactions probably contribute substantially to the etiology of depression. SLEs and especially those occurring early in life are commonly investigated as environmental risk factors for depression. Gene–environment interactions may not necessarily involve genes with a direct association with the disorder or the exposure. However, in order to enhance the power to detect gene–environment interactions a number of strategies have been proposed. Strategies that involve a prioritisation of the genetic polymorphism for testing either by using a gene-disorder or gene–environment association test as a first prioritisation step have been used (20). Gene–environment interaction studies in depression are limited but have included genes involved in the regulation of the HPA axis (20). Previously, an interaction between *CRHR1* and childhood maltreatment has been identified in depression, and the results have subsequently been replicated (as reviewed in Uher et al. (20)). We observed no interaction between genetic variants in *CRHR1* and SLEs. We likewise observed no interaction between SNPs within *ACE* and SLEs. However, a nominally significant interaction between a SNP in *CRH* and SLEs was observed. Given the number of statistical tests in the study and the limited number of individuals, we assume that this interaction is most likely due to chance.

The present study is, to the best of our knowledge, one of the most comprehensive candidate gene studies investigating genes involved in the regulation of the HPA axis for association with depression. Our findings support *ACE* as a potential candidate gene for depression. The study should, however, be viewed in the light of several limitations given the selection of candidate genes and the relatively small sample size. It would be interesting to investigate other genes also involved in the regulation of the HPA axis including the mineral corticoid receptor and other co-chaperones regulating the glucocorticoid receptor and to investigate larger case–control cohorts. Upcoming large genome-wide association studies will likely shed light on common variants associated with depression and reveal whether genes involved in the regulation of the HPA axis influence the risk of depression. In the present study, performing gene–environment interaction analyses was only possible in a subset of the samples. The limited data available decrease the statistical power for the gene–environment interaction analyses and it is thus difficult to assess whether the reported *p*-value represents anything other than a chance finding. However, studies investigating gene–environment interactions in depression are of great relevance since these interactions may potentially explain part of the missing heritability in depression. In the current study, no prioritisation strategy was used and in order to increase power in future gene–environment interactions studies; it may be relevant to consider statistical or biological prioritisation strategies.

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Conflicts of Interest

The authors declare no conflict of interest.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/neu.2016.26

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