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ORIGINAL ARTICLE

Angiotensin-converting enzyme gene variants are associated with both cortisol secretion and late-life depression

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Angiotensin-converting enzyme (ACE) is assumed to influence the activity of the hypothalamic–pituitary–adrenocortical (HPA) axis, which shows hyperactivity in depressed patients. *ACE* could thus be a promising candidate gene for late-life depression but this has not been examined previously. Depression was assessed in 1005 persons aged at least 65 years, at baseline and over the 10-year follow-up. A clinical level of depression (DEP) was defined as having a score of $\geqslant$16 on the Centre for Epidemiology Studies-Depression scale or a diagnosis of current major depression based on the Mini International Neuropsychiatric Interview and according to DSM-IV criteria. Seven single-nucleotide polymorphisms (SNPs) in the *ACE* gene were genotyped and diurnal cortisol secretion, as an index of HPA axis activity, was measured. Multivariable analyses were adjusted for socio-demographic and vascular factors, cognitive impairment, and apolipoprotein E. Strong significant associations were found between all seven SNPs and DEP and, in particular, first-onset DEP in persons without a past history of depression (*P*-values ranging from 0.005 to 0.0004). These associations remained significant after correction for multiple testing. The genotypes that were associated with an increased risk of DEP were also significantly associated with an increase in cortisol secretion under stress conditions. Variants of the *ACE* gene influence cortisol secretion and appear as susceptibility factors for late-life depression in the elderly population. Whether this could represent a common pathophysiological mechanism linking HPA axis and late-life depression remains to be explored.

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INTRODUCTION

Angiotensin-converting enzyme (ACE) converts inactive angiotensin I, into a potent vasoconstrictor and aggravator of endothelial dysfunction, angiotensin II, and degrades the vasodilatator bradykinin, both of which are important in the maintenance of blood pressure.[1] Experimental studies have shown that angiotensin II and ACE can also interfere with the secretion of pituitary hormones such as corticotropin (ACTH) and potentiates the stimulatory effects of corticotropin-releasing hormone (CRH), thus contributing to the stress-related activation of the hypothalamic–pituitary–adrenocortical (HPA) axis.[2,3] Dysregulation of the HPA axis, attested by elevated circulating levels of ACTH and cortisol, is one of the major neuroendocrine abnormalities observed in depression.[4] Cortisol hypersecretion could be a marker of trait vulnerability to depression, which may represent an illness endophenotype.[5,6] In addition, ACE is expressed in various brain regions and is involved in the metabolism of neurokinins that have a role in the transmission of pain, regulation of emotions and alteration of inflammatory and immune responses.[1]

Prior genetic association studies of *ACE* variants and depression have revealed inconsistent results. The most widely studied has been the insertion/deletion (I/D) polymorphism resulting from the presence/absence of a 287-bp fragment in intron 16 of the *ACE* gene. This variant was discovered through its association with ACE plasma levels,[7] but does not appear to be functionally significant[8] and meta-analyses fail to find consistent support for its association with depression.[9,10] However, given that the *ACE* gene is in a region of highly conserved linkage disequilibrium, current opinion favors the presence of other functional polymorphisms in *ACE*.[1,11] In a large study of adults using two independent depression case/control samples, Baghai *et al.*[12] examined associations with 35 *ACE* single-nucleotide polymorphisms (SNPs) and the I/D polymorphism. Only two SNPs, *rs4291* and *rs4295,* were associated with depression in the original sample and *rs4291* was also significant in a replication sample.[12] Only one study involving Thai adults replicated the association between *rs4291* and major depression.[13] Another two found no significant associations[14,15] and another reported inverse associations with closely linked SNPs.[16]

The inconsistencies are likely related to methodological differences including heterogeneity in terms of sample size and study design (family-based[15] vs case-control studies[12–14,16]), the age of the population (childhood,[15] adulthood[12–14,16] or lifetime[17]), depression phenotypes (unipolar and bipolar depression,[15] investigating recurrent episodes,[12,14] or not[13,15–17]), as well as ethnicity (European,[12,14,15,17] Thai[13] or Iranian[16]). Three studies excluded controls with past depression and lifetime anxiety disorder,[12,14,17] but the genome-wide association study was the only one to also exclude controls with current depressive symptomatology.[17] Vascular comorbidity was an exclusion criterion in two studies,[14,16] controlled for in one study,[12] but not considered in two others.[13,17]

Surprisingly, no previous study has focused specifically on the elderly population, despite etiological differences in early and late-onset depression. This includes a later age at onset for vascular depression and specific age-related etiological factors,[18–20] possible higher HPA axis dysfunction and increased cortisol

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response to challenge in the elderly,[21] as well as potential genetic associations between *ACE* and age-related vascular pathologies.[1,22–24] Thus, while there is some evidence to suggest that the *ACE* gene may constitute a susceptibility factor for late-life depression, this hypothesis is yet to be tested within a prospective study in the elderly general population. In this study, we were able to take into account multiple independent and interactive causes of depression, including vascular factors and a history of affective disorder, to help determine the role of the *ACE* gene in late-life depression.

SUBJECTS AND METHODS

Participants

The data were derived from a longitudinal study of neuropsychiatric disorders in community-dwelling French elderly, the Esprit study.[25] Eligible participants, who were at least 65 years of age and non-institutionalized, were recruited by random selection from the electoral rolls between 1999 and 2001. Ethics approval for the study was given by the national ethics committee. After obtaining written informed consent from all participants, interviews were administered by trained staff at baseline and 2, 4, 7 and 10 years of follow-up. Of the 2199 non-demented elderly recruited, participants were excluded from this analysis if they were not assessed for current psychiatric symptomatology ($n=29$) and had missing covariate data ($n=256$). Of the remaining participants, 1005 provided buccal samples for genotyping. Compared with the participants included in the analysis, those excluded had a lower educational level ($\chi^2=13.97$, degree of freedom (df) $=1$, $P=0.0002$), were older ($\chi^2=113.75$, df $=2$, $P<0.0001$) and more likely to have cognitive dysfunction ($\chi^2=55.61$, df $=1$, $P<0.0001$), cardiovascular ischemic pathologies ($\chi^2=33.43$, df $=1$, $P<0.0001$), diabetes ($\chi^2=17.29$, df $=1$, $P<0.0001$), current depressive symptomatology ($\chi^2=26.13$, df $=1$, $P<0.0001$) and to be treated with antidepressant ($\chi^2=14.86$, df $=1$, $P=0.0001$) and antihypertensive drugs ($\chi^2=19.30$, df $=2$, $P<0.0001$).

Outcome measures

The diagnosis of lifetime depression and anxiety disorders was made using the Mini International Neuropsychiatry Interview (MINI), a standardized psychiatric examination validated in the general population[26] according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria.[25] Positive cases were reviewed by a panel of psychiatrists. The Center for Epidemiologic Studies-Depression Scale (CES-D), validated in the elderly, was used to evaluate current depressive symptomatology.[27] Participants with at least one of these criteria at baseline, namely a MINI diagnosis of current major depression or high levels of depressive symptomatology (CES-D$\geqslant$16), were defined as having a clinical level of depression (DEP), that is, levels of psychopathology that would warrant clinical intervention.[28] In longitudinal analysis, participants with prevalent DEP were excluded and incident cases were identified as participants who meet these same criteria during at least one of the four follow-up examinations.

ACE genotyping

The *ACE* gene is located on chromosome 17 and is 21 kb long. *ACE* polymorphisms were chosen based initially on common (MAF$\geqslant$0.05) tag SNPs identified using the Haploview program,[29] and using Caucasian genotype data from the International HapMap Project (http://www.hapmap.org; version3, release R2, ethnicity:CEU + TSI). We then selected SNPs that had been associated with adult or childhood depression in previous studies,[12–17] or those showing significant associations with other vascular-related health outcomes,[1,30,31] while ensuring adequate coverage across the gene (Supplementary Figure S1). There is relatively high linkage disequilibrium across the region estimated using D' (>0.87 across consecutive pairs), although r^2 values are lower. Chosen SNPs include the two most commonly studied, *rs4295* and *rs4311* within intron 2 and 9, and *rs4343* (intron 17), which is known to be strongly associated with the *ACE* I/D polymorphism.[32] The *rs1800764* and *rs4291* SNPs are both in the promoter region, while *rs4333* and *rs4351* are located in intron 16 and intron 19, respectively. The SNPs examined span the *ACE* gene and the 7 SNPs captured 15 of the 23 alleles across the gene at $r^2\geqslant0.80$ of 65%.

DNA was extracted from buccal samples collected during the follow-up as described previously[33] and used to genotype six *ACE* polymorphisms (*rs1800764*, *rs4295*, *rs4311*, *rs4333*, *rs4343* and *rs4351*). Genotyping was performed by LGC Genomics, Hoddesdon, UK using their KASP SNP genotyping system. KASP is a competitive allele-specific PCR incorporating a FRET quencher cassette. The amplified PCR products were analyzed by fluorescence scanning in a BMG labtech Pherastar scanner and the results were interpreted with KlusterCaller 1.1 software. The error rate for the KASP assay system is less than 0.3%. Data concerning an additional *ACE* polymorphism (*rs4291*), as well as replicate data for *rs4343* were also available, as these had been genotyped previously by the Lille Genopole (http://www.genopole-lille.fr/spip/) using DNA extracted from blood samples collected at baseline. The data concerning *rs4343* was identical when we compared the results of genotyping from buccal- and blood-cell-derived DNA, which helped verify the accuracy of the data.

Socio-demographic and clinical variables

The standardized interview included questions on socio-demographic characteristics, height, weight, smoking and alcohol consumption. High blood pressure ($>$140/90 mmHg) and diabetes (fasting glycemia >7 mmol l^{-1} or treated) were recorded. Cognitive function was assessed using the Mini-Mental State Examination and those with score <26 were considered as having cognitive impairment.[34] Detailed medical questionnaires (with additional information from general practitioners) were used to obtain information on history of cardiovascular ischemic pathologies (angina pectoris, myocardial infarction, stroke, cardiovascular surgery, arteritis). We also recorded all drugs used in the preceding month, including antidepressants and antihypertensive drugs. Participants were asked to show medical prescriptions, drug packages and any other relevant information.

Cortisol measurement

HPA activity was evaluated by salivary cortisol in a subsample of participants who were not being treated with medication likely to modify cortisol levels (glucocorticoids, hormonal treatment or benzodiazepines), as described previously.[5] Subjects were instructed not to drink, eat or smoke for at least 30 mn before saliva collection. As cortisol levels increase shortly after awakening, subjects were asked to start the protocol at least 1 h after awakening and subsequently 3, 7 and 14 h after the first morning sampling (the last sampling being collected before midnight to eliminate early cortisol increase occurring during the nocturnal phase) and to record the exact time. As in other naturalistic studies, subjects were allowed to decide wake up and sleep times. Participants were encouraged to carry on their normal daily activities with limited physical exertion to maximize ecological validity. Samples were taken under two contrasting conditions; at the hospital ('stressful situation') just before a lengthy clinical examination, which involved various recognized psychosocial stressors (for example, psychiatric examination, cognitive testing, clinical evaluation and blood collection) and a subsequent quiet day at home with normal daily activities (baseline condition). This thus allowed obtaining maximal contrasting conditions (avoiding novelty or anticipatory effects on baseline conditions measures). Subjects did not report any particular additional stressors on the days they performed sampling. These stress conditions were associated with a highly significant increase in area under the curve (AUC) ($P<0.0001$), as published previously.[5,35] Cortisol levels were determined from saliva collection[36] by direct radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX, USA). Intra-assay and inter-assay coefficients of variation averaged 5%.

Statistical analysis

χ^2 tests were used to compare the distribution of *ACE* genotypes with those predicted under the Hardy–Weinberg equilibrium (HWE). Linkage disequilibrium between the SNPs was calculated using Haploview version 4.2.[29] Associations between *ACE* polymorphisms and prevalent DEP were assessed using logistic regression models. Multivariable logistic regression were adjusted for covariates that were found to be associated with prevalent or incident DEP ($P<0.15$), this included age, gender, education level, cognitive impairment, cardiovascular ischemic pathologies, hypertension, antihypertensive drugs and ApoE. Further adjustment was made for other factors, which were strongly linked to current depression status; a history of past depression and current use of antidepressant, as well as lifetime anxiety disorder. A Cox model with delayed entry was used in the longitudinal analysis of incident DEP over the 10-year follow-up, adjusted for the above confounding factors, and taking age as the basic timescale and birth as the time origin to avoid the problem of nonproportionality of the risk with age.[37]

The association between *ACE* SNPs and cortisol secretion was investigated in the subsample of 259 subjects. As the distribution of raw cortisol is typically skewed, and the normal diurnal profile may be approximated by an exponential curve, cortisol values were log-transformed. Given the nonfixed time-sampling protocol, cortisol levels were calculated at fixed times from the regression of the four-cortisol values on the sampling times, for each participant and on two different days (basal and stressful situation). AUC were standardized and calculated between 0800 hours and 2200 hours for each subject (extrapolating values from the equation of the regression line), as published previously.[5,35] The associations between *ACE* SNPs and cortisol AUC were evaluated using analysis of covariance and adjusting for age, gender and DEP. SAS (v9.1, SAS Institute, NC, USA) was used for the statistical analyses with a significance level of $P<0.05$. Given that seven SNPs were investigated, the Bonferroni-corrected *P*-value was 0.0071.

RESULTS

Population characteristics

At baseline, 25.4% of the 1005 participants were identified as having DEP and antidepressant use was reported by 4.2% of persons (Table 1). Depressed persons were more frequently women, had a lower education level, were more likely to have cognitive dysfunction and a history of major depression, and more frequently carried the ApoE-ε2 allele, than nondepressed persons. The *ACE* genotype frequencies, shown in Table 2, were not significantly different from those predicted by HWE in the whole sample and among the nondepressed control participants ($P>0.28$ for all SNPs).

ACE polymorphisms and prevalent DEP

All SNPs were found to be significantly associated with the risk of DEP at baseline in logistic regression model adjusted for age and gender (Supplementary Table S1) as well as in multi-adjusted logistic regression model (Table 2). The comparison of homozygotes was highly significant (OR between 0.44 and 0.54) and remained significant after Bonferroni correction. For *rs4291* and *rs4295*, there was a weaker significant association for the heterozygotes also. The same highly significant associations were found after further adjusting for a history of major depression and antidepressant use, as well as lifetime anxiety disorder (data not shown). Interactions were observed between several *ACE* polymorphisms and a history of past major depression (Table 3). After stratification by past major depression and further adjusting for antidepressant use, strong significant associations were found between all seven SNPs and the risk of DEP, for participants without a past history of depression (*P*-values 0.0004–0.001) but not for the 24.7% of participants who reported past major depression.

As antidepressant treatment could have an effect on the level of DEP, we also performed a sensitivity analysis by changing the definition of DEP to also include participants not reaching our criteria but currently using antidepressants. Very similar highly significant associations were found (Supplementary Table S2).

Table 1. Baseline characteristics of participants according to prevalent depression[a] ($n=1005$)

Characteristic	*Nondepressed* (n = *750*)	*Depressed* (n = *255*)	*Depressed* vs *nondepressed*	*Wald test*
	%	%	*OR (95% CI)*[b]	P-*value*
Age (years)				
65–69	39.20	36.47		0.20[c]
70–74	38.13	35.29		
75+	22.67	28.24		
Gender: women	55.07	72.94	—	<0.0001[c]
⩾12 years of schooling	30.67	17.25	0.54 (0.38–0.78)	0.001
Cardiovascular ischemic pathologies[d]	10.53	10.59	1.14 (0.71–1.83)	0.60
Antihypertensive drugs				
None	61.60	60.39	—	—
ACE inhibitor	16.67	21.18	1.34 (0.92–1.95)	0.13
Other	21.73	18.43	0.81 (0.55–1.19)	0.28
Blood pressure⩾140/90 mm Hg	50.13	45.49	0.90 (0.67–1.21)	0.48
Diabetes[e]	6.84	5.88	1.12 (0.61–2.06)	0.72
At least one ApoE-ε2 allele	83.60	89.02	1.57 (1.01–2.45)	0.05
Cognitive impairment (MMSE<26)	8.40	15.69	1.84 (1.20–2.84)	0.006
Antidepressant use	1.87	10.98	5.55 (2.85–10.8)	<0.0001
History of major depression	20.53	36.86	2.03 (1.47–2.79)	<0.0001

Abbreviations: ApoE, apolipoprotein E; CES-D, Center for Epidemiologic Studies-Depression Scale; MMSE, Mini-Mental State Examination. [a]Corresponds to current major depression or a CES-D score⩾16. [b]Adjusted for age and gender. [c]χ^2 test.
[d]A history of angina pectoris, myocardial infarction, stroke, cardiovascular surgery, arteritis. [e]Fasting glycemia levels > 7 mmol l^{-1} or treated ($n=1001$ as four subjects had missing data).

ACE polymorphisms and incident DEP

In longitudinal analyses, among the 750 elderly without DEP at baseline, 167 (22.3%) had incident DEP over the 10-year follow-up period (median (IQR) = 8.9 (7.7–9.0) years). Across all participants in this sample and in the group without incident depression, the *ACE* genotype frequencies were not significantly different from those predicted by the HWE (Table 4). In Cox models adjusted for age and gender, participants heterozygous for *rs4291* and *rs4295* had a reduced risk of incident DEP over follow-up ($P=0.006$ and $P=0.009$, respectively). After multivariable adjustment, these associations reached traditional but not corrected significance levels. We also examined incident depression cases defined as participants with DEP or those using antidepressants over the follow-up period ($n=736$, 193 events). The associations actually increased in significance ($P<0.003$ for heterozygous of *rs4291* and *rs4295*; and $P=0.06$ for *rs4311*) (Supplementary Table S3).

ACE polymorphisms and diurnal cortisol secretion

In models adjusted for age, gender and DEP, no significant associations were found between any of the *ACE* SNPs and cortisol AUC under basal conditions (Table 5). In contrast, under stressful conditions, we found a highly significant association between *rs4291* and *rs4311* and AUC. For another four SNPs, the *P*-values were significant at uncorrected levels, while for *rs1800764,* there was only a trend-level association ($P=0.09$). The direction of these associations was in line with the findings from the DEP analysis, with the reference homozygote group (AA for *rs4291*, *rs4343* and *rs4351* and CC for *rs4295*, *rs4311* and *rs4333)* having the highest cortisol levels, and the heterozygous genotype having intermediate levels. The same significant associations were found in models further adjusted for cardiovascular ischemic diseases, hypertension or antihypertensive drug use, history of major depression or anxiety disorder (data not shown).

DISCUSSION

As the first prospective study to examine the association between variants in the *ACE* gene and clinical levels of depression in the elderly, we report that all the seven polymorphisms across the *ACE* gene examined were strongly associated with both cortisol hypersecretion and late-life depression. Two SNPs were also associated with the risk of incident DEP over the 10-year follow-up. The consistency of associations reported here contrasts with prior work examining adult depression, where associations predominantly being reported with individual SNPs only, and

Table 2. Multi-adjusted logistic regression analysis for the association between *ACE* polymorphisms and prevalent depression[a]

SNP and genotype	*Nondepressed*		*Depressed*		*OR (95% CI)*[b]	P
	n	%	n	%		
rs1800764 (n = *983*)						
TT	197	26.88	85	34.00	—	—
CT	354	48.29	123	49.20	0.81 (0.58–1.15)	0.24
CC	182	24.83	42	16.80	0.53 (0.34–0.81)	0.004
rs4291 (n = *977*)						
AA	242	33.33	109	43.43	—	—
AT	350	48.21	114	45.42	0.74 (0.54–1.02)	0.07
TT	134	18.46	28	11.16	0.44 (0.27–0.71)	0.001
rs4295 (n = *998*)						
CC	244	32.66	108	43.03	—	—
CG	365	48.86	114	45.42	0.72 (0.52–0.99)	0.04
GG	138	18.47	29	11.55	0.45 (0.28–0.72)	0.001
rs4311 (n = *963*)						
CC	149	20.64	67	27.80	—	—
CT	342	47.37	124	51.45	0.83 (0.57–1.19)	0.31
TT	231	31.99	50	20.75	0.47 (0.30–0.72)	0.001
rs4333 (n = *933*)						
CC	130	18.73	59	24.69	—	—
CT	331	47.69	123	51.46	0.85 (0.58–1.26)	0.42
TT	233	33.57	57	23.85	0.54 (0.35–0.83)	0.006
rs4343 (n = *982*)						
AA	126	17.17	55	22.18	—	—
AG	352	47.96	129	52.02	0.82 (0.55–1.21)	0.32
GG	256	34.88	64	25.81	0.54 (0.35–0.84)	0.006
rs4351 (n = *976*)						
AA	128	17.63	58	23.20	—	—
AG	342	47.11	128	51.20	0.81 (0.55–1.18)	0.27
GG	256	35.26	64	25.60	0.53 (0.34–0.81)	0.003

Abbreviations: ACE, angiotensin-converting enzyme; CES-D, Center for Epidemiologic Studies-Depression Scale; CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism. [a]Corresponds to current major depression or a CES-D score ⩾16. [b]Model adjusted for age, gender, education, cardiovascular ischemic pathologies, cognitive impairment, ApoE2, antihypertensive drugs, and high blood pressure.

Table 3. Multi-adjusted logistic regression analysis for the association between *ACE* polymorphisms and prevalent depression[a] according to history of major depression

SNP and genotype	*P-interaction*	*Without history of major depression (n = 757)*		*With history of major depression (n = 248)*	
		OR (95% CI)[b]	P	*OR (95% CI)*[b]	P
rs1800764					
TT	0.08	—	—	—	—
CT		0.69 (0.45–1.05)	0.08	1.21 (0.65–2.24)	0.54
CC		0.41 (0.24–0.70)	0.001	1.17 (0.52–2.64)	0.70
rs4291					
AA	0.14				
AT		0.67 (0.44–1.00)	0.05	0.97 (0.54–1.72)	0.91
TT		0.36 (0.20–0.65)	0.0007	1.08 (0.42–2.78)	0.88
rs4295					
CC	0.06	—	—	—	—
CG		0.61 (0.41–0.91)	0.01	1.07 (0.60–1.93)	0.82
GG		0.34 (0.19–0.62)	0.0004	1.16 (0.46–2.93)	0.75
rs4311					
CC	0.05	—	—	—	—
CT		0.61 (0.39–0.95)	0.03	1.68 (0.84–3.38)	0.15
TT		0.39 (0.23–0.66)	0.0004	0.94 (0.40–2.24)	0.89
rs4333					
CC	0.17	—	—	—	—
CT		0.70 (0.43–1.14)	0.15	1.30 (0.64–2.61)	0.47
TT		0.46 (0.27–0.79)	0.005	1.02 (0.45–2.31)	0.97
rs4343					
AA	0.01	—	—	—	—
AG		0.56 (0.35–0.90)	0.02	2.02 (0.94–4.36)	0.07
GG		0.40 (0.23–0.67)	0.0005	1.51 (0.64–3.56)	0.35
rs4351					
AA	0.08	—	—	—	—
AG		0.61 (0.38–0.98)	0.04	1.49 (0.72–3.10)	0.28
GG		0.42 (0.25–0.71)	0.001	1.18 (0.51–2.71)	0.70

Abbreviations: ACE, angiotensin-converting enzyme; CES-D, Center for Epidemiologic Studies-Depression Scale; CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism. [a]Corresponds to current major depression or a CES-D score ⩾16. [b]Model adjusted for age, gender, education, cardiovascular ischemic pathologies, cognitive impairment, ApoE2, high blood pressure, antihypertensive and antidepressant drugs.

the specific variants differing across studies. Our findings were independent of potential confounders, for example, socio-demographic, mental and physical health, including vascular factors.

Prior studies have focused predominantly on the *ACE* I/D polymorphism but they have yielded inconsistent results.[9,10] Recent evidence indicates that this variant is not functional,[8] but may be a marker for a yet unidentified functional variant. However, the power to detect significant associations between a marker locus such as the I/D variant and a causal variant is dependent on both the linkage disequilibrium between the variants and the sample size.[38] This could thus help explain the discordance in previous findings. It has been shown that there may be several promoter and intragenic *ACE* variants, which influence ACE levels,[39] with a suggestion that the regions between intron 18 and the 3′UTR, as well as the 5′-UTR being potential key areas.[1] We found that SNPs across the entire gene were associated with DEP, despite some of them being in only moderate linkage disequilibrium measured using the more appropriate r^2 (cf. Supplementary Figure S1B).[40] The strongest associations were observed with *rs4291*, located in the promoter region, and the closely linked *rs4295* in intron 2. The importance of variation in or around the promoter regions has been suggested in a number of common diseases[41] and functional consequences for *ACE* gene transcription regulation has been proposed for *rs4291*.[12] Furthermore, this SNP does appear to be functionally significant as it has been associated with ACE level in two studies.[42,43] While no prior study has examined the functionality of the other variants examined in this study, we used a specific program RESCUE-ESE (http://genes.mit.edu/burgelab/rescue-ese/)[44] to help predict the effects of single-base changes on exonic splicing enhancer activity and thus their potential functionality. Our analysis indicated that several of the SNPs can affect alternative splicing (*rs4291* and *rs4295* can affect, as can *rs4311* and *rs4351*), suggesting their potentially important functional role. The other SNPs could also possibly be functionally important as they have been associated with other health-related outcomes in prior studies[31,42,45] (see also http://www.alzgene.org/geneoverview.asp?geneID=125), although this remains unknown.

Interestingly, *rs4291* and *rs4295* were also the two variants identified as being most strongly associated with recurrent major depression in a previous study of 35 *ACE* SNPs,[12] but surprisingly the associations were in the reverse direction. The reason for this difference is unknown, but the possibility of population stratification cannot be ignored. In the earlier study, these SNPs were not in HWE in their control sample, which may be suggestive of genotyping errors.[46] To overcome this issue, the authors selected another 'epidemiological' control sample and confirmed that HWE was adhered to in this population. However, with these controls, no significant associations were found with either *rs4291* or *rs4295*.[12] Another explanation relates to their focus on recurrent depression in adults, whereas our study was on late-life

Table 4. Multi-adjusted Cox proportional hazards analysis for the association between *ACE* polymorphisms and 10-year incidence of depression[a]

SNP and genotype	*Nondepressed*		*Depressed*		*HR (95% CI)*[b]	P
	n	%	n	%		
rs1800764 (n = *733*)						
TT	149	26.19	48	29.27	—	
CT	284	49.91	70	42.68	0.84 (0.58–1.23)	0.38
CC	136	23.90	46	28.05	1.14 (0.75–1.71)	0.54
rs4291 (n = *726*)						
AA	175	31.03	67	41.36	—	
AT	287	50.89	63	38.89	0.66 (0.46–0.93)	0.02
TT	102	18.09	32	19.75	0.88 (0.57–1.35)	0.55
rs4295 (n = *747*)						
CC	177	30.52	67	40.12	—	
CG	298	51.38	67	40.12	0.67 (0.48–0.95)	0.03
GG	105	18.10	33	19.76	0.89 (0.58–1.36)	0.58
rs4311 (n = *722*)						
CC	108	19.35	41	25.00	—	
CT	277	49.64	65	39.63	0.75 (0.50–1.11)	0.14
TT	173	31.00	58	35.37	0.94 (0.63–1.41)	0.78
rs4333 (n = *694*)						
CC	98	18.22	32	20.51	—	
CT	269	50.00	62	39.74	0.84 (0.54–1.29)	0.42
TT	171	31.78	62	39.74	1.15 (0.75–1.77)	0.53
rs4343 (n = *734*)						
AA	94	16.43	32	19.75	—	
AG	284	49.65	68	41.98	0.81 (0.53–1.24)	0.33
GG	194	33.92	62	38.27	0.99 (0.64–1.52)	0.95
rs4351 (n = *726*)						
AA	97	17.08	31	19.62	—	
AG	277	48.77	65	41.14	0.84 (0.54–1.29)	0.42
GG	194	34.15	62	39.24	1.03 (0.67–1.60)	0.89

Abbreviations: ACE, angiotensin-converting enzyme; CES-D, Center for Epidemiologic Studies-Depression Scale; CI, confidence interval; HR, hazard ratio; SNP, single-nucleotide polymorphism. [a]Corresponds to current major depression or a CES-D score ⩾16. [b]Cox model with age as the timescale and adjusted for gender, education, cardiovascular ischemic pathologies, cognitive impairment, ApoE2, antihypertensive drugs, and high blood pressure.

Table 5. Association between *ACE* polymorphisms and cortisol secretion (*n* = 259)

SNP and genotype	*Basal cortisol AUC*			*Stress cortisol AUC*		
	P	*Mean*[a]	*s.e.*[a]	P	*Mean*[a]	*s.e.*[a]
rs1800764						
TT	0.91	63.13	0.93	0.09	66.43	0.79
CT		63.14	0.80		64.90	0.68
CC		62.64	1.12		63.99	0.96
rs4291						
AA	0.21	64.25	0.86	0.006	67.13	0.73
AT		63.11	0.82		64.34	0.71
TT		61.67	1.34		64.24	1.12
rs4295						
CC	0.48	63.94	0.87	0.02	66.92	0.74
CG		63.32	0.81		64.85	0.70
GG		62.15	1.31		63.71	1.11
rs4311						
CC	0.08	65.03	1.10	0.004	67.86	0.95
CT		63.51	0.79		65.14	0.68
TT		61.72	1.09		63.69	0.94
rs4333						
CC	0.26	65.08	1.16	0.02	67.73	1.01
CT		63.36	0.81		65.20	0.70
TT		62.66	1.08		64.11	0.93
rs4343						
AA	0.44	64.33	1.11	0.04	67.37	0.95
AG		63.91	0.80		65.69	0.69
GG		62.67	0.99		64.26	0.85
rs4351						
AA	0.51	64.50	1.13	0.02	67.69	0.96
AG		63.70	0.78		65.52	0.68
GG		62.83	1.01		64.18	0.87

Abbreviations: ACE, angiotensin-converting enzyme; AUC, area under the curve; CES-D, Center for Epidemiologic Studies-Depression Scale; SNP, single-nucleotide polymorphism. [a]Adjusted for age, gender and depression (corresponding to current major depression or a CES-D score ⩾16).

depression, with highly significant associations found in participants without a past major depression only. Depression is known to be a heterogeneous and multi-factorial disease likely to be caused by a number of environmental, biological and genetic factors with varying interactions across the lifespan.[20,47] Different etiologies have been reported for initial and recurrent depression[48,49] as well as for early and late-onset depression,[18,19] including vascular depression.[20] Given the higher dysfunction of the HPA axis and increased cortisol response to challenge in the elderly,[21] as well as the potential genetic associations between *ACE* and age-related vascular pathologies,[1,22–24] it seems conceivable that certain *ACE* genes may be specifically associated with late-life depression.

Another consideration is that several studies focused entirely on major depression without screening for depressive symptoms. The consequence is that 'controls' themselves may actually have high levels of depressive symptoms. Only in the GAIN study did the authors report that controls with depressive symptoms were excluded from their analyses and they reported a nominally significant association between *rs4333* and major depression in the same direction as ours[17] but in the reverse direction to that of Baghai *et al.*[12] Our data highlights the importance of controlling for lifetime major depression in the whole sample, as well as taking into account the potential for current depressive symptomatology in the control group.

In terms of 10-year incident DEP, the association with *rs4291* and *rs4295* was significant in the comparison of heterozygotes versus homozygotes only. This suggests a heterosis effect, which may actually occur in up to 50% of all gene association studies and particularly those involving Europeans.[50] Heterosis has been reported with certain *ACE* polymorphisms for longevity,[51] blood pressure response to ACE inhibitor[52] and Alzheimer's disease[32] and other chronic disorders.[53,54] Several explanations have been proposed,[50] which includes an independent third factor causing a hidden stratification of the sample such that the depressive phenotype would be associated with either one set of homozygote subjects or the alternate homozygosity set. This may also include interactions with other genes, environmental factors or age-related incident factors such as vascular factors.[50] However, in our analysis, we observed the same results after excluding participants with prevalent and incident vascular ischemic pathologies during the follow-up, as well as those with incident dementia. Another explanation is based on an inverted U-shaped response curve in which either too little or too much gene expression is deleterious, with optimal gene expression occurring in heterozygotes. The exact reason for our observation of a heterosis effect with incident DEP only, remains unclear.

Limitations to our study include the bias introduced from excluding participants with missing data, who were in poorer

health and more likely to be diagnosed with depression during follow-up, thus reducing the overall power of the study. In terms of ethnicity, French law prohibits us from questioning participants about this directly, however prior genotyping data from a subsample of these participants indicates that less than 1% is non-Caucasian (unpublished data and Lambert *et al.*[55]). This is further supported by similarity between the *ACE* genotype frequencies observed in our population, with those published previously in white Europeans[12,13,45,56] (and http://www.alzgene.org). It remains possible that other unknown confounding factors may have influenced the associations, including subclinical disease, which was not detectable through the analysis of lipids, glycemia and hypertension. However, it is unlikely that the presence of vascular disorders has influenced our results; associations remained significant after controlling for vascular factors or excluding persons with ischemic pathologies. Although this study in the elderly population was limited to four salivary cortisol measures on 2 days, the basal characteristics of cortisol secretion are similar to previous studies with more frequent sampling.[57] The random selection of subjects, the systematic return of saliva samples by all the subjects and the choice of a nonfixed time-sampling protocol (known to improve compliance particularly in the elderly[58,59]) also suggests cortisol findings to be representative of naturalistic conditions.

Our study is strengthened by its design and population-based sample. This is the first prospective study of late-life depression to investigate potential associations with several *ACE* polymorphisms in community-dwelling elderly, with a strong *a priori* biological rationale. The findings of genetic associations of *ACE* polymorphisms with cortisol secretion further support our results concerning DEP. DEP was assessed by trained staff using two distinct measures validated in the general population, including a structured diagnostic interview[26,27] according to DSM-IV criteria. We controlled for a large number of covariates, thus minimizing any confounding, which contrasts with previous studies that have predominantly presented minimally adjusted analyses in clinical samples. Finally, in addition to using a genotyping system with a low error rate, we also controlled for the accuracy of *ACE* genotyping by comparing the genotyping data obtained from blood and cheek DNA extracts for one SNP.

Several experimental and human studies have demonstrated a strong interrelationship between angiotensin II and the secretion of pituitary hormones, which may influence the susceptibility to develop late-life depression.[2,20,47] Our findings provide strong epidemiological support for the involvement of *ACE* polymorphisms in late-life depression. These variants were also linked to cortisol hypersecretion, which is a typical marker of depression. We cannot exclude that in addition, other hormones (for example, angiotensin and aldosterone) not examined in this study and other related genetic factors could also be associated with depression. Future longitudinal studies are needed to unravel the mechanisms involved, by determining whether this could represent a common pathophysiological mechanism linking the HPA axis and later-life depression. Future work could also assess the utility of *ACE* polymorphisms and this phenotypic outcome (cortisol hypersecretion) as a marker of late-life depression prognosis and progression.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DISCLAIMER

The funders had no role in the design and conduct of the study; in data collection, management, analysis, interpretation of the data; or writing the report preparation, review or approval of the manuscript.

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