

# 11 $\beta$ -Hydroxylase (*CYP11B1*) gene variants and new-onset depression in later life

Marie-Laure Ancelin, PhD; Joanna Norton, PhD; Karen Ritchie, PhD;  
Isabelle Chaudieu, PhD; Joanne Ryan, PhD

**Background:** Cumulative exposure to high glucocorticoid levels is detrimental for the brain and may have particular implications in later life. A feature of late-life depression is increased cortisol secretion. Variants in the *CYP11B1* gene, which codes for the enzyme responsible for cortisol synthesis, could influence risk of late-life depression, but this hypothesis has not been examined. We investigated the associations between variants in the *CYP11B1* gene and late-life depression, taking into account history of depression and potential sex-specific effects. **Methods:** We assessed depression in 1007 community-dwellers aged 65 years or older (60% women) at baseline and over a 14-year follow-up. A clinical level of depression was defined as a score of  $\geq 16$  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 the criteria of the *Diagnostic and Statistical Manual of Mental Disorders, 4th edition* (DSM-IV). We examined incident and recurrent depression in participants without or with a history of major depression, respectively. We genotyped 5 single-nucleotide polymorphisms (SNPs) spanning *CYP11B1*. We used multivariable analyses to adjust for age, body mass index, cardiovascular ischemic pathologies, hypertension, cognitive impairment and anxiety. **Results:** In women, *rs6471580* and *rs7016924* were associated with a 50% lower rate of incident (new-onset) late-life depression, and *rs11783855* was associated with a 2.4-fold higher rate of late-life depression. These associations remained after correction for multiple testing, but we found no associations for recurrent depression in women or men. **Limitations:** This study focused on the major gene involved in corticosteroid biosynthesis, but other genes may also be implicated in this pathway. **Conclusion:** Variants of the *CYP11B1* gene appear to be susceptibility factors for late-life depression in a sex-specific manner.

## Introduction

Depression is an etiologically and genetically heterogeneous mood disorder, and interactions between age at onset and various molecular variants have been suggested.<sup>1,2</sup> In older adults, late-life depression encompasses both late-onset cases and early-onset cases that recur or continue into the later years of life;<sup>3</sup> the 2 types differ with respect to symptoms, etiology and genetic susceptibility.<sup>1,2,4</sup>

One of the most consistent findings in depression is altered activity of the hypothalamic–pituitary–adrenal (HPA) axis involving enhanced cortisol release in concert with reduced feedback sensitivity.<sup>5,6</sup> Cortisol secretion can be influenced by a number of factors or moderators, such as age, sex, comorbidity and genetic sensitivity to environmental stress.<sup>5,7–10</sup> With increasing age, the HPA axis might become more vulnerable to dysregulation,<sup>5,11</sup> given the accumulation of lifetime stressful events and age-associated neurobiological changes.<sup>4,12</sup> Female sex hormones can influence depression<sup>13</sup> and the HPA axis response to stress.<sup>14</sup> Women exhibit higher prevalence of depression<sup>12</sup> and stronger responses to stress,

as well as immune and inflammatory reactions,<sup>14</sup> especially in older adults.<sup>11</sup> Cortisol and depression heritability are high (over 40%),<sup>7,15</sup> and there is clear evidence for female specificity in the genetic basis for this.<sup>16,17</sup> Sex-specific associations between genes for glucocorticoid signalling and the response of the HPA axis to stress have also been reported.<sup>18</sup>

There is some clinical and genetic evidence to suggest that major depression can be divided into a subtype that is more vulnerable to intrinsically stress-related environmental factors (e.g., reactive depression) and a subtype with a strong biological and/or genetic basis and no apparent environmental precipitants (e.g., endogenous).<sup>15,19,20</sup> In an older population, we reported neuroendocrine heterogeneity in late-life depression, with differential stress-related susceptibility according to genetic variability. Those homozygous for the short allele of the serotonin transporter gene-linked polymorphic region (5-HTTLPR) showed a cortisol-related neuroendocrine vulnerability to late-onset depression with no apparent environmental precipitants (endogenous depression), whereas those homozygous for the long allele were more likely to have recurrent depression and greater vulnerability

**Correspondence to:** M.L. Ancelin, Inserm U1061, Hôpital La Colombière, 39 avenue Flahault, BP 34493, 34093 Montpellier Cedex 5, France; marie-laure.ancelin@inserm.fr

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to stressful life events (reactive depression).<sup>21,22</sup> Variants of the angiotensin-converting enzyme (*ACE*) gene known to interfere with the functioning of the HPA axis also appeared as susceptibility factors for late-onset depression (but not recurrent depression), and they also influenced cortisol secretion.<sup>23</sup>

The steroid 11 $\beta$ -hydroxylase (encoded by the cytochrome P450 family 11 subfamily B member 1 *CYP11B1* gene) is the terminal enzyme of glucocorticoid production responsible for the conversion of deoxycortisol to cortisol and also of 11-deoxycorticosterone to corticosterone.<sup>24</sup> Cortisol, the major glucocorticoid in humans, is principally produced in the adrenal gland, but also in the heart and vascular system, and expression of 11 $\beta$ -hydroxylase has been reported in the brain.<sup>24</sup> Despite the fact that cortisol clearly plays a role in several stress-related psychiatric disorders — especially depression — the possible influence on depression of genes involved in corticosteroid biosynthesis is still poorly explored. Previous candidate-gene association studies have focused on the central regulation of the HPA axis (e.g., genes related to corticotropin-releasing hormone or corticosteroid signalling).<sup>25</sup>

Although there is some evidence to suggest that the *CYP11B1* gene may constitute a susceptibility factor for late-life depression, this hypothesis has yet to be tested in a prospective study in the older general population. In this study, we took into account sex differences and multiple independent and interactive causes of depression, including vascular and neuropsychological factors. This study also considered the history of previous major depression to clarify the role of the *CYP11B1* gene in late-onset depression versus recurrent depression in the elderly.

## Methods

### Participants

Community-dwelling participants 65 years and older were recruited by random selection from the electoral rolls between 1999 and 2001 as part of the prospective ESPRIT study of neuropsychiatric disorders in the older French general population.<sup>26</sup> The protocol was approved by the national ethics committee (Ethical Committee of Sud Méditerranée III and University Hospital of Kremlin-Bicêtre, France), and written informed consent was obtained from each participant. Interviews were administered by trained staff at baseline and at 2- to 3-year intervals for 14 years. Of the 2161 participants without dementia assessed for current psychiatric symptoms in the ESPRIT study, 1060 provided buccal samples for genotyping and had at least 1 follow-up. Of those, 42 had missing data on past major depression and 11 on other key covariates. Compared to the 1007 participants included in the analysis, those excluded had a lower educational level, were older and had higher frequencies of cardiovascular ischemic pathologies, hypertension, cognitive impairment, depression and antidepressant use ( $p \leq 0.001$ ).

### Outcome measures

Lifetime depression and anxiety disorders were diagnosed using the Mini-International Neuropsychiatry Interview

(MINI), a validated standardized psychiatric examination,<sup>27</sup> according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV).<sup>26</sup> Positive cases were reviewed by a panel of psychiatrists. Current depressive symptoms were evaluated using the Center for Epidemiologic Studies Depression Scale (CES-D), validated in an older general population.<sup>28</sup> Participants with a MINI diagnosis of current major depression or high levels of depressive symptoms (CES-D  $\geq 16$ ) at baseline were defined as having a level of depression that would warrant clinical intervention.<sup>29</sup> In longitudinal analysis, participants with prevalent depression were excluded, and incident cases were defined as those who met the criteria above during at least 1 of the 6 follow-up examinations. Participants with incident dementia were also excluded.

### CYP11B1 genotyping

The 11 $\beta$ -hydroxylase enzyme participates in steroidogenesis and is encoded by the *CYP11B1* gene, which is localized on 8q24.3, has 9 exons and spans 6593 base pairs (142872.4K to 142879.8K, GRCh38.p13). We chose *CYP11B1* polymorphisms based on common (minor allele frequency  $\geq 0.05$ ) tag SNPs identified using Haploview (version 4.2),<sup>30</sup> and Caucasian genotype data from the International HapMap Project (www.hapmap.org; version 3, release R2, ethnicity CEU + TSI [Utah residents with northern and western European ancestry + Tuscans from Italy]). Chosen SNPs were *rs11783855* (142801.5, 3' untranslated region [UTR]), *rs1134096* (142872.8, gene region), *rs7011830* (142901.0, 5' UTR), *rs6471580* (142905.3, 5' UTR) and *rs7016924* (142924.7, 5' UTR; Appendix 1, Figure S1, available at [jpn.ca/190177-a1](http://jpn.ca/190177-a1)). Genotyping was performed from buccal DNA by LGC Genomics using the KASP SNP genotyping system as described previously.<sup>31</sup> The error rate for the KASP assay system is less than 0.3%.

### Sociodemographic and clinical variables

The standardized interview included questions about socio-demographic characteristics, lifestyle and physical health. High blood pressure ( $> 160/95$  mm Hg), diabetes (fasting glycemia  $\geq 7$  mmol/L or treated), weight and height were recorded, and body mass index ( $\text{kg}/\text{m}^2$ ) was calculated. Cognitive impairment was defined as a score less than 26 on the Mini-Mental State Examination.<sup>32</sup> Detailed medical questionnaires (with additional information from general practitioners) provided information about participants' history of cardiovascular ischemic pathology (angina pectoris, myocardial infarction, stroke, cardiovascular surgery or arteritis). We also recorded all drugs used in the preceding month, including antidepressants. Dementia was diagnosed at each follow-up by a neurologist as part of a standardized examination, and validated by a panel of independent neurologists.<sup>33</sup>

### Statistical analysis

We used  $\chi^2$  tests to compare the distribution of *CYP11B1* genotypes with those predicted under Hardy-Weinberg equilibrium. We calculated linkage disequilibrium between

the SNPs using Haploview.<sup>30</sup> We performed multinomial logistic regression analyses to compare 3 groups using a general genotype model: participants with current depression but without a history of major depression (late-onset), participants with current depression and past major depression (recurrent), and participants without depression. We assessed associations between *CYP11B1* polymorphisms and late-onset or recurrent depression in men and women using logistic regression adjusted for age. We used multivariate models to further adjust for health-related variables that could be associated with depression and/or *CYP11B1* mutations:<sup>34</sup> obesity, hypertension, cardiovascular pathologies, cognitive impairment and current anxiety disorder.

In the longitudinal analysis of incident depression over the 14-year follow-up, we used a Cox model adjusted for the above potential confounding factors, with delayed entry and age as the time scale. The use of age as the time scale is deemed more appropriate for time-to-event analysis in the elderly population: rather than modelling time to incident depression based on when a person is recruited for the study, it models their hazard of depression based on their age.<sup>35,36</sup> As a consequence, observations are left-truncated and right-censored, so an extension of the standard proportional hazards model is required that incorporates delayed entries. We used SAS (version 9.4, SAS Institute, Inc.) for the statistical analyses, with significance set at  $p < 0.05$ . Because we investigated 5 SNPs, the Bonferroni-corrected value was  $p < 0.01$ .

## Results

### Population characteristics

At baseline, 256 of the 1007 participants had depression, of whom 37.1% had recurrent depression and 62.9% had late-

onset depression. Comparing the frequencies between the 3 groups, we observed that participants with recurrent or late-onset depression were more likely to be women, to have cognitive impairment or a current anxiety disorder, and to take antidepressant medication (Table 1). Compared to participants without depression, participants with recurrent depression were more likely to be obese, and those with late-onset depression were more likely to have a lower education level. Among participants with depression, those with recurrent depression were more likely to be women, to take antidepressant medication and to have a current anxiety disorder than those who did not have past major depression.

### CYP11B1 polymorphisms and prevalent depression

The *CYP11B1* genotype frequencies were not significantly different from those predicted under Hardy–Weinberg equilibrium ( $p > 0.05$  for all SNPs). In men in the age-adjusted logistic regression model, none of the 5 SNPs was significantly associated with either late-onset or recurrent depression (Appendix 1, Table S1). In women, heterozygotes of *rs7011830*, *rs6471580* and *rs7016924* were associated with approximately 50% decreased odds of late-onset depression (Table 2). These associations were also significant in the multivariate adjusted logistic regression model, and for 2 of the SNPs, even at the Bonferroni-corrected significance level. The findings were the same when our definition of depression included participants who did not have current symptoms but were taking antidepressants (Appendix 1, Table S2).

### CYP11B1 polymorphisms and incident depression

In longitudinal analyses, of the 689 participants (311 men and 378 women) without depression at baseline, 201 (29.2%) had

**Table 1: Baseline characteristics of participants according to depression status\* and history of major depression (n = 1007)**

Characteristics, n (%)†	No current depression (n = 751)	Current depression and history of major depression (n = 95)	Current depression and no history of major depression (n = 161)	Global p value‡
Age, yr (mean ± SD)	71.41 ± 4.39	71.85 ± 4.42	72.04 ± 4.51	0.24¶
Female	414 (55.13)	76 (80.00)	112 (69.57)	< 0.001¶
Education ≥ 12 yr	231 (30.76)	20 (21.05)	24 (14.91)	0.002
Body mass index				
Normal (< 25 kg/m <sup>2</sup> )	427 (56.86)	54 (56.84)	88 (54.66)	
Overweight (25–29 kg/m <sup>2</sup> )	275 (36.62)	30 (31.58)	59 (36.65)	
Obese (≥ 30 kg/m <sup>2</sup> )	49 (6.52)	11 (11.58)	14 (8.70)	0.37
Diabetes (fasting blood glucose ≥ 7.0 mmol/L or treated)	51 (6.84)	4 (4.21)	11 (6.83)	0.76
Cardiovascular ischemic pathology§	78 (10.39)	9 (9.47)	18 (11.18)	0.79
Hypertension (> 95/160 mm Hg or treated)	335 (44.61)	41 (43.16)	71 (44.10)	0.97
Cognitive impairment (MMSE score < 26)	62 (8.26)	15 (15.79)	25 (15.53)	0.017
Antidepressant use	15 (2.00)	15 (15.79)	13 (8.07)	< 0.001
Current anxiety disorder	70 (9.32)	34 (35.79)	29 (18.01)	< 0.001

CES-D = Center for Epidemiologic Studies—Depression; MMSE = Mini-Mental State Examination; SD = standard deviation.

\*Current major depression or a CES-D score ≥ 16.

†Unless otherwise specified.

‡Calculated from multinomial logistic regression adjusted for age (continuous) and sex.

§History of angina pectoris, myocardial infarction, stroke, cardiovascular surgery or arteritis.

¶Calculated from multinomial logistic regression adjusted for sex or age (continuous).

**Table 2: Logistic regression analysis for the association between CYP11B1 polymorphisms and prevalent depression according to history of major depression in women**

SNP and genotype	Risk of recurrent depression in later life (n = 191)					Risk of late-onset depression (n = 411)				
	No depression	Depression*	OR (95% CI)†	p value	OR (95% CI)‡	No depression	Depression*	OR (95% CI)†	p value	OR (95% CI)‡
<i>rs11783855</i> , n	115	74	—	—	—	294	108	—	—	—
GG, %	23.48	29.73	—	—	—	28.23	27.78	—	—	—
GT, %	53.04	47.30	0.73 (0.36–1.47)	0.37	0.72 (0.34–1.53)	46.26	43.52	0.96 (0.56–1.63)	0.87	0.98 (0.57–1.69)
TT, %	23.48	22.97	0.77 (0.33–1.77)	0.53	0.69 (0.28–1.71)	25.51	28.70	1.15 (0.64–2.08)	0.64	1.09 (0.60–1.99)
<i>rs1134096</i> , n	115	75	—	—	—	294	107	—	—	—
TT, %	31.30	36.00	—	—	—	32.65	36.79	—	—	—
GT, %	45.22	42.67	0.82 (0.42–1.61)	0.57	0.79 (0.39–1.61)	53.06	39.62	0.66 (0.40–1.09)	0.10	0.66 (0.39–1.10)
GG, %	23.48	21.33	0.80 (0.36–1.79)	0.59	0.66 (0.28–1.56)	14.29	23.58	1.45 (0.78–2.69)	0.24	1.47 (0.78–2.77)
<i>rs7011830</i> , n	107	72	—	—	—	285	104	—	—	—
CC, %	31.78	36.11	—	—	—	32.28	40.38	—	—	—
AC, %	43.93	45.83	0.91 (0.46–1.80)	0.79	0.92 (0.44–1.90)	54.39	37.50	0.55 (0.33–0.91)	0.021	0.55 (0.33–0.92)
AA, %	24.30	18.06	0.66 (0.29–1.55)	0.34	0.55 (0.22–1.38)	13.33	22.12	1.32 (0.70–2.49)	0.39	1.36 (0.71–2.60)
<i>rs6471580</i> , n	115	70	—	—	—	291	108	—	—	—
AA, %	29.57	24.29	—	—	—	20.62	30.56	—	—	—
AG, %	43.48	52.86	1.46 (0.71–3.02)	0.31	1.90 (0.85–4.24)	56.01	37.96	0.46 (0.27–0.79)	0.005	0.43 (0.25–0.75)
GG, %	26.96	22.86	0.99 (0.42–2.30)	0.98	1.22 (0.49–3.01)	23.37	31.48	0.91 (0.50–1.65)	0.76	0.83 (0.45–1.54)
<i>rs7016924</i> , n	113	75	—	—	—	296	110	—	—	—
GG, %	32.74	26.67	—	—	—	23.65	31.82	—	—	—
AG, %	43.36	49.33	1.39 (0.69–2.78)	0.36	1.60 (0.75–3.40)	55.41	39.09	0.52 (0.31–0.89)	0.016	0.49 (0.28–0.83)
AA, %	23.89	24.00	1.22 (0.54–2.75)	0.64	1.56 (0.65–3.72)	20.95	29.09	1.04 (0.57–1.87)	0.91	0.92 (0.50–1.69)

CES-D = Center for Epidemiologic Studies-Depression; CI = confidence interval; MMSE = Mini-Mental State Examination; OR = odds ratio; SNP = single nucleotide polymorphism.

\*Current major depression or a CES-D score  $\geq 16$ .

†Adjusted for age (continuous).

‡Adjusted for age, ischemic pathologies, body mass index, hypertension, cognitive impairment (MMSE &lt; 26) and current anxiety.

incident depression over the follow-up period (median 15.3 [interquartile range 4.1] years). In men in Cox multivariate models adjusted for age, none of the 5 SNPs was significantly associated with incident depression (data not shown). In contrast, we found significant associations in women without a history of major depression. Compared with major homozygotes, minor homozygotes of *rs11783855* were associated with a 136% higher rate of incident depression, and heterozygotes of *rs6471580* and *rs7016924* were associated with a rate of incident depression that was more than 50% lower (Table 3).

## Discussion

This was the first prospective study to investigate the association between variants of the 11 $\beta$ -hydroxylase gene and clinical levels of depression in older adults. Variation in the *CYP11B1* gene was strongly associated with prevalent and incident late-onset depression in women specifically. Our findings were independent of potential physical and mental health-related confounders, supporting the critical role of corticosteroid biosynthesis and the HPA axis in new-onset late-life depression.

To our knowledge, no prior candidate-gene study has investigated potential associations between 11 $\beta$ -hydroxylase polymorphisms and current depression in later life. Gerritsen and colleagues<sup>37</sup> examined the possible influence of 41 *CYP11B1* SNPs on lifetime diagnosis of major depressive disorder in the adult general population and did not report any highly significant associations. However, controls in their study were not screened for depressive symptoms, and they did not consider potential differences between men and women.

Depression is a heterogeneous and multifactorial disease, likely to be caused by a number of environmental, biological and genetic factors with varying interactions across the lifespan.<sup>38,39</sup> Different etiologies have been reported for initial and recurrent depression,<sup>40,41</sup> as well as for early- and late-onset depression.<sup>1,4</sup> The results of recent genome-wide association studies also suggest different genetic susceptibilities according to age at onset<sup>2</sup> and etiologic heterogeneity within major depression as a function of environmental adversities.<sup>42</sup>

In the ESPRIT population, we have already reported neuroendocrine heterogeneity in late-life depression, with differential stress-related susceptibility according to genetic variability. The short allele homozygotes of 5-HTTLPR showed cortisol-related neuroendocrine



**Table 3: Multi-adjusted Cox proportional hazards analysis for the association between CYP11B1 polymorphisms and 14-year incidence of depression according to history of major depression in women**

SNP and genotype	Risk of incident recurrent depression (n = 109)				Risk of incident late-onset depression (n = 269)			
	No depression	Depression*	HR (95% CI)†	p value	No depression	Depression*	HR (95% CI)†	p value
<i>rs11783855</i> , n	56	53			174	90		
GG, %	16.07	30.19	—	—	32.18	23.33	—	—
GT, %	55.36	50.94	0.68 (0.35–1.33)	0.26	52.30	34.44	0.97 (0.55–1.69)	0.91
TT, %	28.57	18.87	0.60 (0.27–1.37)	0.23	15.52	42.22	2.36 (1.37–4.05)	0.002
<i>rs1134096</i> , n	56	53			175	89		
TT, %	32.14	30.19	—	—	32.00	33.71	—	—
GT, %	44.64	47.17	1.09 (0.55–2.16)	0.81	56.57	46.07	0.74 (0.46–1.19)	0.21
GG, %	23.21	22.64	1.17 (0.53–2.56)	0.70	11.43	20.22	1.81 (1.00–3.27)	0.05
<i>rs7011830</i> , n	55	56			169	88		
CC, %	30.91	32.61	—	—	31.36	34.09	—	—
AC, %	45.45	43.48	1.03 (0.49–2.14)	0.95	57.99	47.73	0.73 (0.45–1.18)	0.20
AA, %	23.64	23.91	1.26 (0.55–2.89)	0.58	10.65	18.18	1.73 (0.93–3.19)	0.08
<i>rs6471580</i> , n	56	53			173	89		
AA, %	28.57	30.19	—	—	16.76	28.09	—	—
AG, %	44.64	43.40	0.80 (0.41–1.56)	0.51	60.12	49.44	0.42 (0.25–0.69)	0.0007
GG, %	26.79	26.42	0.70 (0.32–1.50)	0.36	23.12	22.47	0.58 (0.32–1.05)	0.07
<i>rs7016924</i> , n	56	51			176	90		
GG, %	30.36	33.33	—	—	20.45	31.11	—	—
AG, %	44.64	45.10	0.80 (0.42–1.54)	0.51	58.52	48.89	0.46 (0.28–0.75)	0.002
AA, %	25.00	21.57	0.64 (0.28–1.46)	0.29	21.02	20.00	0.60 (0.33–1.10)	0.10

CES-D = Center for Epidemiologic Studies-Depression; CI = confidence interval; HR = hazard ratio; MMSE = Mini-Mental State Examination; SNP = single nucleotide polymorphism.

\*Current major depression or a CES-D score  $\geq 16$  (37.8% incidence).

†Cox model with age as timescale and adjusted for ischemic pathologies, body mass index, hypertension, cognitive impairment (MMSE < 26) and current anxiety disorder, as well as for history of major depression for the entire sample of women.

vulnerability to late-onset depression with no apparent environmental precipitants (endogenous depression); long allele homozygotes were more reactive to stressful life events and more likely to have recurrent depression (reactive depression).<sup>21,22</sup> We also showed that variants of the *ACE* gene were susceptibility factors for late-onset but not recurrent depression, and they also influenced cortisol secretion.<sup>23</sup> In the present study, we found that genetic vulnerability in *CYP11B1*, a gene involved in cortisol production, was associated with higher risk of late-onset but not recurrent depression. Our findings suggest that several genetic factors related to neuroendocrine pathways and cortisol secretion might shape late-onset depression in older adults.

Age and sex have been reported to modify the cortisol response to challenge. A meta-analysis reported a consistent effect of age on cortisol responses that was almost 3-fold stronger in older women than in older men.<sup>11</sup> Female-specific genetic determinants of morning cortisol levels have also been reported in a genome-wide study.<sup>17</sup> Sex hormones can influence the HPA axis response to stress, and a more potent reaction to stress has been observed in women.<sup>14</sup> This finding may help explain, at least in part, the higher prevalence of late-life depression in women and the sex-specific vulnerability. Finally, estrogen-related receptor  $\alpha$  (ERR $\alpha$ ) has been reported to act as a transcriptional activator of *CYP11B* genes, and may also be involved in the sex-specific nature of our findings.<sup>43</sup>

In the present study, the SNP associated with a 2.4-fold increased rate of late-life depression (*rs11783855*) was located in the 3' UTR of the *CYP11B1* gene; for *rs6471580* and *rs7016924*, located in the 5' UTR, heterozygotes were associated with a

50% decreased rate of late-onset depression. Some mutations in the *CYP11B1* gene have been associated with hypertension, familial hyperaldosteronism and impaired production of cortisol and steroid precursors.<sup>34,44</sup> The functionality of the variants in our study has not been examined directly, but the associations with depression remained significant after controlling for hypertension and vascular factors. With these SNPs, Velders and colleagues<sup>45</sup> reported nonsignificant associations with basal salivary diurnal cortisol secretion in the older general population, but they did not specifically consider women and late-onset depression. However, *rs11783855* could have functional significance, because it is found in the 3' UTR regulatory region, which plays a key role in controlling gene expression. The importance of variation in or around the promoter regions has also been suggested in a number of common diseases.<sup>46</sup> Moreover, some SNPs could be very far from the causative gene, as reported for some steroid biosynthesis- or cytochrome-P450-related genes.<sup>47</sup> In humans, *CYP11B1* is highly homologous with and lies only 40 kb downstream from *CYP11B2*, which codes for the isozyme aldosterone synthase, catalyzing the 2 last steps of aldosterone (the main human mineralocorticoid) production.<sup>34</sup> This *CYP11B2/CYP11B1* locus is polymorphic and displays relatively tight linkage disequilibrium.<sup>48</sup> A chimeric gene comprising the 5' regulatory region of *CYP11B1* and the 3' coding region of *CYP11B2* has been reported to cause primary hyperaldosteronism.<sup>49</sup> Thus, *rs6471580* is located in the 5' UTR of *CYP11B1* but also in the 3' UTR of *CYP11B2*. The functional consequence of these variants on 11 $\beta$ -hydroxylase/aldosterone synthase remains to be examined.

## Limitations

Study limitations included bias from excluding participants with missing data; they were in poorer health and more likely to be depressed during follow-up, thus reducing the overall study power. Potential bias from population stratification needs to be considered, because French law prohibits collecting data related to ethnicity. However, previous genotyping data from these participants indicated that more than 99% were White Europeans.<sup>23</sup> This was supported by the similarity of the *CYP11B1* genotype frequencies with those published previously for White Europeans ([www.ncbi.nlm.nih.gov/projects/SNP/](http://www.ncbi.nlm.nih.gov/projects/SNP/)). Other unknown factors could have influenced the associations, including subclinical disease, which was not detectable from measurement of blood pressure, lipids and glycemia. However, it is unlikely that the presence of vascular disorders and neuropsychiatric comorbidity influenced our results, because associations remained significant after controlling for these factors.

Strengths of our study were its design and the large population-based sample. This was the first prospective study of late-life depression to investigate potential associations with *CYP11B1* polymorphisms in community-dwelling older men and women separately over 14 years, with a strong a priori biological rationale. Depression was assessed by trained staff using 2 distinct measures validated in the general population, including a structured lifetime diagnostic interview based on DSM-IV,<sup>27,28</sup> and considering late-onset depression specifically. A large amount of health data were collected, which allowed us to control for potential confounding or mediating factors, and we were able to consider sex differences. The genotyping system used in our study had a very low error rate, and we chose 5 SNPs to ensure satisfactory coverage across the gene.

A special consideration is that our study focused on a specific candidate gene, rather than using a genome-wide association study approach. Candidate-gene studies are hypothesis-driven and are of value for investigating known genes with strong a priori biological rationale; they also help to reduce the risk of false positives, especially in smaller studies. The genome-wide association study approach involves a large sample but merely flags genomic regions without a direct link to underlying biological functions. Given that genome-wide association studies need to use very large samples, they almost always use more “crude” measures of depression, based on self-report or electronic records. Candidate-gene studies can use very tight phenotypic definitions and specific subclassifications, such as recurrent versus late-onset depression. Candidate-gene studies and genome-wide association studies have generally failed to implicate replicable gene variants.<sup>50</sup> There are several reasons for these discrepancies. Depression is etiologically and phenotypically heterogeneous, further complicated by its mixed course (ranging from a single lifetime episode to chronic recurrent disorder) and frequent psychiatric comorbidity. Above all, it is now largely accepted that depression has multiple causal pathways involving numerous genetic loci with small additive effects, and possible gene × gene or gene × environment interactions. Together, this could explain why, despite considerable efforts and very large samples, genome-wide association studies of depression have failed until very recently to yield replicable

genetic loci.<sup>51</sup> The next challenges will be to identify causal variants and reveal the genetic architecture of depression, its subtypes and its broader phenotypes.

## Conclusion

Our findings provide strong epidemiological support for *CYP11B* polymorphisms as sex-specific independent susceptibility factors for late-life depression. They support our hypothesis of individual neuroendocrine heterogeneity and depression subtypes and may help provide an explanation for clinical heterogeneity in response to treatment.<sup>52</sup> Clinical trials based on HPA activation by stress and antigluco-corticoid augmentation therapy appeared inefficient in adult patients with chronic treatment-resistant (i.e., recurrent) depression but efficacious in patients who were not selected for treatment resistance.<sup>21</sup>

However, depression is a complex trait; it is likely that in addition to the effects of single genetic variants, depression also is likely to be influenced by gene × environment and gene × gene interactions. Although there is consistent evidence in epidemiological studies for the physiologic and clinical relevance of variants in this HPA-axis-regulating gene, large longitudinal studies are needed to replicate our original findings. Such studies should aim to establish the causal mechanism behind these associations, combining genetic and steroid level data (e.g., basal and stress acute or long-term cortisol secretion, aldosterone) with additional HPA axis markers (e.g., DNA methylation, gene expression) in diverse populations, taking into account factors such as age, age of depression onset, sex and ethnicity. Comprehensive functional genomic studies will also be needed before drawing definite conclusions about the role of 11β-hydroxylase in late-life depression.

**Affiliations:** From Inserm, Université Montpellier, Neuropsychiatry: Epidemiological and Clinical Research, Montpellier, France (Ancelin, Norton, Ritchie, Chaudieu, Ryan); the Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, United Kingdom (Ritchie); and the Biological Neuropsychiatry and Dementia Unit, School of Public Health and Preventive Medicine, Monash University, Melbourne, Victoria, Australia (Ryan).

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