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DNA methylation and genetic variation of the angiotensin converting enzyme (*ACE*) in depression

Dilys Lam[a], Marie-Laure Ancelin[b], Karen Ritchie[b,c], Richard Saffery[a], Joanne Ryan[a,b,d,*]

[a] Cancer & Disease Epigenetics, Murdoch Children's Research Institute, Royal Children's Hospital & Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia
[b] INSERM, U1061, Neuropsychiatrie, Recherche Clinique et Epidémiologique, Univ. Montpellier, Montpellier, France
[c] Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK
[d] Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria, Australia

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ABSTRACT

Background: Depression is one of the most prevalent psychiatric disorders, and in older persons is associated with high levels of comorbidity and under-treatment. Dysfunction of the hypothalamic-pituitary-adrenal (HPA) stress axis is consistently observed in the older population as well as depressed patients, with the angiotensin converting enzyme (*ACE*) a key regulator of the stress response. Epigenetic regulation of *ACE* may play an important role in HPA axis (dys)regulation.
Objective: To investigate *ACE* promoter methylation as a biomarker of late-life depression, and its association with genetic variation and cortisol secretion.
Method: The longitudinal general population ESPRIT study is aimed at investigating psychiatric disorders in older persons (n = 1863, average age = 73). Depression was assessed using the Mini International Neuropsychiatric Interview according to DSM-IV criteria and the Centre for Epidemiologic Studies Depression Scale (CES-D). Genotype information for seven polymorphisms across the *ACE* gene was also available. Blood and saliva samples collected at baseline and used to extract DNA and measure cortisol, respectively. Sequenom MassARRAY was used to measure promoter DNA methylation of the *ACE* gene (n = 552).
Results: There was no evidence of an association between *ACE* promoter methylation and depression. However, there was evidence that *ACE* genetic variants influenced methylation, and modified the association between depression and methylation (Δ at various sites; -2.05% to 1.74%; p = 0.019 to 0.039). Multivariate analyses were adjusted for participants' lifestyle, health and medical history. Independent of depression status, *ACE* methylation was inversely correlated with cortisol levels (r = −0.336, p = 0.042).
Conclusion: This study provides evidence that associations between *ACE* methylation and depression are genotype-dependent, suggesting that the development of reliable depression biomarkers may need to consider methylation levels in combination with underlying genetic variation. *ACE* methylation may also be a suitable biomarker of cortisol and/or HPA axis activity.

1. Introduction

Depression is one of the most prevalent psychiatric disorders, yet despite considerable advances in clinical practice, case detection remains relatively poor at all ages. In older persons, untreated depression has been furthermore associated with both high rates of functional impairment and comorbidity, notably cardiovascular disease (CVD) (Alexopoulos, 2005). One of the principal factors contributing to poor case identification is insufficient knowledge of the mechanisms underlying disease onset, and in particular the causes of variations in clinical presentation and treatment response. Development of specific biomarkers would significantly contribute to a better understanding of disease etiology and heterogeneity, as well as improving case detection and monitoring.

Growing evidence suggests that depression results from complex interactions between genetic and environmental factors (Caspi et al., 2003; Booij et al., 2013). Epigenetic mechanisms such as DNA methylation may play an important role due to their ability to respond to both genetic and environmental influences to potentiate changes in gene activity (Tsankova et al., 2007). Whilst depression is a brain-based disorder, the effects on an individual are more wide-spread, and physiological changes in peripheral tissues have been observed (O'Donovan

* Corresponding author at: Department of Epidemiology and Preventive Medicine Monash University Level 5, The Alfred Centre 99 Commercial Road Melbourne VIC 3004 Australia.
E-mail address: joanne.ryan@monash.edu (J. Ryan).

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et al., 2010; Thomson et al., 2014). Furthermore, brain tissue cannot be accessed in living humans, so human epigenetic marks have been predominantly studied in peripheral tissues including blood and buccal (mouth epithelial cells) (Lockwood et al., 2015), which offer an advantage of being easily accessible, thus contributing to their potential as biomarkers. Biomarkers do not always lie along causal pathways, but must robustly associate with the phenotype to ensure diagnostic utility.

The hypothalamic-pituitary-adrenal (HPA) axis is a neuroendocrine system that is central to regulating responses to stress (Palazidou, 2012). In older persons, a high dysfunction of the HPA axis is observed (Otte et al., 2005) which may be enhanced by stimulatory effects of genes involved in the axis, such as that coding for the angiotensin converting enzyme (*ACE*). *ACE* converts Angiotensin I into Angiotensin II to regulate cardiovascular homeostasis (Sayed-Tabatabaei et al., 2006). *ACE* also has a well-characterised role in regulating the HPA axis following stress-activation through stimulatory effects on two hormones, adrenocorticotropin hormone (ACTH) and corticotropin-releasing-hormone (CRH)(Armando et al., 2007; Pavlatou et al., 2008). Hyperactivity of the axis is observed consistently in depressed patients, as attested by elevated levels of cortisol, CRH and ACTH (Lloyd and Nemeroff, 2011). *ACE* is notably considered a candidate susceptibility factor for the bi-directional relationship between depression and CVD (Bondy, 2007). Genetic studies have linked several *ACE* genetic variants to an increased risk of depression (Baghai et al., 2006; Lopez-Leon et al., 2008; Ancelin et al., 2013) and to antidepressant treatment response (Baghai et al., 2001; Baghai et al., 2004). *ACE* polymorphisms have also been shown to regulate cortisol secretion, a measure of HPA axis activity (Baghai et al., 2002, 2006; Ancelin et al., 2013).

To date, few studies have investigated DNA methylation of *ACE* and its association with depression. This study used data gathered from a large population based cohort of older individuals to investigate the associations between depression status and methylation at the promoter region of the *ACE* gene in peripheral blood. Analyses were adjusted for participants' lifestyle, health and medical history. Potential modifying effects of genetic variation across the *ACE* gene and cortisol levels were also considered.

2. Methods

2.1. Study participants

The ESPRIT study is a French longitudinal general population study assessing neuropsychiatric disorders in older individuals (Ritchie et al., 2004). Participants were recruited by random selection from electoral rolls within the Montpellier district, between 1999 and 2001. Non-institutionalised individuals aged 65 years and over were eligible and were contacted via mail with an invitation to participate, along with information detailing study procedures. The ESPRIT study protocol was approved by the regional ethics committee (Ethical Committee of University Hospital of Kremlin-Bicêtre, France). Following recruitment, participants underwent standardised clinical assessments, as well as health and psychiatric interviews. Information was collected on the participants' lifestyle, present state of health, medical history, medication use and exposure to adverse environmental factors. All participants provided written informed consent.

2.2. Depression assessment

Current major depressive disorder (MDD) was diagnosed using a standardised psychiatric interview; the Mini International Neuropsychiatric Interview (MINI, French version 5.00), following the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) (American Psychiatric Association, 1994). The MINI has been widely used in clinical and research settings, and validated within the general population setting (Sheehan et al., 1998). Participants that met the DSM-IV criteria for MDD were further reviewed by a panel of three psychiatrists and a psychologist with access to information from other health assessments. The Centre for Epidemiologic Studies-Depression (CES-D) scale was also administered to assess the severity of depressive symptoms (Radloff, 1977). This questionnaire has been previously validated in the older population (Radloff, 1977; Beekman et al., 1997). In contrast to the MINI, the CES-D scale is a self-reporting questionnaire, with a score of 16 or above considered the threshold for significant depressive symptoms warranting further clinical investigation(Radloff, 1977). To take into account that late-life depression covers a range of mild to severe depressive symptoms(Fiske et al., 2009), late-life depression assessment was based on two criteria; having a current diagnosis of MDD, or severe depressive symptoms (CES-D $\geq$ 16), in keeping with prior work (Ancelin et al., 2013; Januar et al., 2015).

2.3. Genotyping

Of the 1863 recruited participants, 785 agreed to provide blood samples at baseline for research. Genomic DNA was extracted from white blood cells following a standard protocol(Ritchie et al., 2009), with samples stored at −80 °C until use. Genotyping was performed by LGC Genomics, Hoddesdon, UK using their KASP SNP genotyping system (Ancelin et al., 2013). This included data for seven single nucleotide polymorphisms (SNPs) across the *ACE* gene: *rs1800764, rs4291, rs4295, rs4311, rs4333, rs4343, rs4351*. These variants were chosen based on prior associations with depression and to ensure adequate coverage across the gene (Ancelin et al., 2013). The distribution of genotypes was examined using a chi-squared test for Hardy-Weinberg equilibrium. Linkage disequilibrium between the SNPs were also calculated, with relatively high linkage disequilibrium across 3 SNPs (*rs1800764, rs4291*, *rs4295*) as estimated by r^2 values (> 0.75) (Ancelin et al., 2013).

2.4. ACE promoter methylation assay

A 356 base pair (bp) assay (hg_38: chr17: 63476511–63476866) was designed using the Epidesigner package (epidesigner.com) to cover a region of the *ACE* promoter CpG island, spanning 32 CpG sites, where differential methylation of several CpG sites was previously reported (Fig. **S1**) (Zill et al., 2012). Sodium bisulphite conversion of 500 ng DNA from each participant blood sample was performed using the EZ-96 DNA Methylation-Lightning MagPrep Kit (Zymo Research; Irvine, USA) following the manufacturer's protocol. PCR amplification of the region of interest was performed in technical triplicates for all participant samples (Table S1), to account for possible variability (Coolen et al., 2007). The primers used were F: 5′-TAGAGGAAGTTGGAGAAA GGGTT-3′ and R: 5′- CCTACAAAACACCTAAAATCCCC-3′, with an adapter (5′-aggaagagag) and T7 tag (5′-cagtaatacgactcactatagggagaaggct) attached respectively. DNA methylation was quantified for each triplicate sample using the SEQUENOM MassARRAY EpiTYPER platform (Coolen et al., 2007). Mean methylation values were calculated from replicates within 10% of the median (Martino et al., 2013). Further quality control included the exclusion of CpG units or participants where less than 50% of methylation data was available, to help ensure that spurious data was not analysed. Clearly outlying data points, i.e. those more than 3 times the interquartile range, were also excluded (n = 21). Following this quality control, data was obtained for 16 CpG units encompassing 25 CpG sites (Table S2, Fig. S1).

2.5. Cortisol measurement

Salivary cortisol was collected from a subsample of participants not taking medications likely to affect cortisol levels(Ancelin et al., 2013) and measured by direct radioimmunoassay (Diagnostic systems Laboratories, Webster, TX, USA) (Hellhammer, 1987). Cortisol samples were obtained at four time points throughout the day. Participants were told to withhold from eating, drinking or smoking at least 30 min before