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Methylation of the glucocorticoid receptor gene associated with depression in patients with acute coronary syndrome

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ABSTRACT

Objective: The present study investigated the longitudinal effects of *NR3C1 1 F* exon methylation on the risk of depression following ACS and treatment outcomes.

Methods: In total, 969 patients admitted for recent ACS were recruited within 2 weeks of ACS; 711 of these patients were followed up at 1 year. Depressive disorder was diagnosed according to DSM-IV criteria and included prevalent depressive disorder at baseline and incident or persistent depressive disorder at follow-up based on depression status at the two examinations. Of the 378 baseline participants who were diagnosed with depression, 255 participated in a randomized double-blind placebo-controlled trial of escitalopram, while the remaining 123 were managed with the usual medical treatment for ACS.*NR3C1 1 F* exon methylation was measured using peripheral blood samples, and various demographic and clinical characteristics were assessed as covariates.

Results: Higher *NR3C1 1 F* exon methylation levels were independently associated with prevalent depressive disorder at baseline but not with incident or persistent depressive disorder at follow-up based on logistic regression analyses adjusted for covariates. The effects of escitalopram on the remission of depressive symptoms was not influenced by *NR3C1 1 F* exon methylation status in ACS patients, but a placebo effect on the remission of depressive symptoms was observed, particularly in patients with lower methylation levels.

Conclusions: ACS patients with higher *NR3C1 1 F* exon methylation levels were at higher risk of developing depressive disorder within 2 weeks of ACS. Additionally, adequate antidepressant treatment may be effective for the remission of depressive symptoms regardless of *NR3C1 1 F* exon methylation status.

1. Introduction

Because acute coronary syndrome (ACS) is a leading cause of death, increased effort has been made toward improving its treatment outcomes and prognosis (Benjamin et al., 2018). Depression is a common comorbidity in ACS patients and is associated with poor prognoses with increased morbidity and mortality (Lichtman et al., 2014). These phenomena may be due to common biological mechanisms shared by ACS and depression. Because depression can be treated, a clearer understanding of the pathophysiology of depression in ACS patients could improve its prognosis.

Several putative biological mechanisms have been suggested to account for depression in ACS patients, including inflammation, autonomic dysregulation, and endothelial dysfunction – all of which play roles in depression and ACS (Stapelberg et al., 2011; Granville Smith et al., 2015). Our study group investigated the genetic roles of the serotonin pathway (Kim et al., 2015a), brain-derived neurotrophic fator (Kim et al., 2015b; Kang et al., 2015), inflammation including tumor

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necrosis factor (TNF)-α (Kim et al., 2016), and interleukin (IL)-1β (Kang et al., 2017) in depression after ACS. Furthermore, dysregulation of the hypothalamus–pituitary–adrenal (HPA) axis, which is crucial in controlling the stress response, may be involved in the development of depression after ACS. Disruption of the HPA axis has been implicated in the risk of cardiovascular disease via changes in the inflammatory cytokines and body composition (Girod and Brotman, 2004; Nijm and Jonasson, 2009). Moreover, HPA axis dysregulation is significantly associated with the pathophysiology of depression (Pariante and Lightman, 2008).

However, the HPA axis has yet to be explored in depth as a biological mechanism underlying depression in ACS patients even though this system is greatly involved in the stress response and ACS constitutes a fundamental psychological and physiological stressor that results in depression in some ACS patients. Previous studies investigating the association between cortisol and depression in ACS patients have produced inconsistent findings. For example, one study observed positive associations between 24-hour urinary cortisol levels and depression in chronic ACS patients (Otte et al., 2004), another found a flatter diurnal profile in suspected ACS patients with depression (Bhattacharyya et al., 2008), and still another found no association between cortisol output and depression 4 months after ACS (Molloy et al., 2008). It is possible that these inconsistencies are due to the diurnal rhythm of cortisol as well as differences in cortisol assessment methods, use of fluid samples, and assessment time after ACS.

Attempts to elucidate the inconsistent findings from previous studies of cortisol have suggested that glucocorticoid receptors (GRs) may be a possible candidate for research on HPA dysregulation because the effects of cortisol are primarily mediated by GRs (Alt et al., 2010). GR dysfunction contributes to impaired negative feedback in the HPA axis, which in turn leads to alterations in HPA axis function (Pariante and Lightman, 2008). In fact, previous studies of HPA dysregulation that focused on ACS patients with depression found that decreased levels of GR mRNA were implicated in this condition (Nikkheslat et al., 2015). The expression of GRs is affected by various genetic polymorphisms of the GR gene (Spijker and van Rossum, 2012) as well as by epigenetic modifications (de Kloet et al., 2005). With respect to the genetic vulnerability of GRs, a common GR haplotype that includes the minor allele of the 9beta A/G polymorphism is associated with insufficient glucocorticoid signaling, which may increase the risk of depression in patients with chronic coronary heart disease (Otte et al., 2009). However, the association between GR polymorphisms and depression in ACS remains inconclusive due to the lack of studies investigating depression in ACS patients as well as inconsistent findings regarding the associations between GR polymorphisms and ACS (Otte et al., 2010; Koeijvoets et al., 2008) or depression (Zobel et al., 2008; Szczepankiewicz et al., 2011).

Similarly, no studies have investigated the association between epigenetic alterations and depression in ACS patients. Epigenetic changes in GRs might be a promising candidate for a biological marker of depression following ACS due to its role in the regulation of gene expression without changing the DNA sequence (Murgatroyd and Spengler, 2011) and because it can be modified by environmental stress (Weaver et al., 2004). High levels of methylation of the GR gene, known as nuclear receptor subfamily 3, group C, member 1 (*NR3C1*) promoter and exon *1 F*, is correlated with reductions in GR expression similar to typical DNA methylation in other genes (Perroud et al., 2014). Several previous studies have shown that *NR3C1* promoter and exon *1 F* hypermethylation are associated with depression in the general population (Nantharat et al., 2015; Roy et al., 2017) as well as with atherosclerosis and cardiovascular reactivity including blood pressure that were closely related to physiological response to stress (Zhao et al., 2015; Li-Tempel et al., 2016). Therefore, it is possible that altered *NR3C1 1 F* methylation might predispose individuals to the development of depression and influence the response to antidepressant treatments in those who suffer from unexpected medical illnesses that might trigger stress responses, such as ACS events. Therefore, the present study aimed to investigate whether the methylation status of the *NR3C1 1 F* region would be associated with depression at baseline and at 1 year after ACS and with the treatment response to depression using data from large prospective studies, including naturalistic and interventional studies, of ACS patients.

2. Materials and methods

2.1. Study overview and participants

The present analyses were conducted using data from a large prospective study of ACS patients known as the Korean DEPression in ACS (K-DEPACS) study. The K-DEPACS study included a nested interventional randomized 24-week double-blind placebo-controlled trial of escitalopram named the Escitalopram for DEPression in ACS (EsDEPACS) study (clinicaltrials.gov registry number: NCT00419471). The details and overall designs of both studies have been published previously (Kim et al., 2014) and the study outline and recruitment process are delineated in Supplementary Figure 1. All participants who took part in the baseline assessments were asked to complete a follow-up assessment at 1 year after ACS to estimate the incidence and persistence of depression. Written informed consents for both studies were obtained, and both were approved by the Chonnam National University Hospital Institutional Review Board.

2.2. The K-DEPACS baseline study

The K-DEPACS study was intended to examine the epidemiology of depressive disorder in ACS patients using a naturalistic prospective design. Participants were successively enrolled from among patients who were hospitalized at the Department of Cardiology of Chonnam National University Hospital, Gwangju, South Korea, to manage recent ACS (N = 4809). All ACS patients who fulfilled the eligibility criteria and not the exclusion criteria (detailed in the Supplementary material), and who agreed to participate in the K-DEPACS study (N = 1152) were evaluated for depressive symptoms within 2 weeks of the ACS event (mean 6.3 ± 2.4 days). Of the 1152 participants, 969 consented to blood sampling and comprised the baseline sample.

2.2.1. Evaluation of depression

To evaluate the association between *NR3C1 1 F* methylation at baseline and depression after ACS longitudinally, depression was assessed by study psychiatrists using DSM-IV diagnostic criteria within 2 weeks after ACS and at 1 year after ACS using the Mini-International Neuropsychiatric Interview (MINI; Sheehan et al., 1998). Based on these criteria, major depressive disorders were diagnosed in patients with at least one core symptom (i.e., depressed mood or loss of interest) and at least 4 other symptoms of depression, whereas minor depressive disorders were diagnosed in patients with 2–4 symptoms in total including at least one core symptom. For the present study, ACS patients with a depressive disorder were considered those who had either minor or major depressive disorder. At baseline and follow-up, depressive disorder status was classified as three binary variables: prevalent depressive disorder, defined as the presence of depressive disorders at baseline; incident depressive disorder, defined as the absence of a depressive disorder at baseline but its presence at follow-up; and persistent depressive disorder, defined as the presence of a depressive disorder both at baseline and at follow-up. Additionally, the severity of depressive symptoms was evaluated using the Hamilton Depression Rating Scale (HAMD; Hamilton, 1960).

2.2.2. NR3C1 methylation

The DNA methylation status of the *NR3C1 1 F* gene was evaluated using venous blood samples obtained at the baseline assessments. Exon *1 F* has been the focus of most previous studies investigating *NR3C1*

methylation because it includes a binding site for nerve growth factor-inducible protein A (NGFI-A), which is highly expressed in the hippocampus of pups with nurturing mothers (McCormick et al., 2000). Hence, this region has been studied in humans, particularly by studies investigating its association with early adverse experiences (Martin-Blanco et al., 2014; Perroud et al., 2011). Similar to previous studies, we investigated the methylation patterns in the *NR3C1* (GeneBank #AY 436590) exon *1 F* region, specifically three CpG sites in exon *1 F* (Supplementary Fig. 2), which correspond to CpG 1–3 in the study by Perroud et al. (2011), CpG 40–42 in the study by Palma-Gudiel et al. (2015), and CpG sites 45–47 in the study by Daskalakis and Yehuda (2014). We selected these sites based on their prominence in previous studies and because their methylation statuses were associated with adverse life experiences and related disorders, including depression (Perroud et al., 2011, 2014; Martin-Blanco et al., 2014; Bustamante et al., 2016). Moreover, the relatively consistent direction of the association between methylation and adverse experiences was discussed in a recent review article (Daskalakis and Yehuda, 2014).

The detailed methods of examination for the three CpG sites are described in the Supplementary materials and in recent publications (Kang et al., 2018). Briefly, the three investigated CpG sites (described in Supplementary Fig. 2) were located in the CpG-rich region of *NR3C1* exon *1 F* between -3166 and -3147 (CGGTGGCCCTCTTAACGCCG) relative to the translational start site (+1). In the present analyses, the individual methylation percentages at the three CpG sites and their average values were utilized.

2.2.3. Demographic and clinical characteristics at baseline

Several characteristics that could potentially act as confounding variables of the relationship between depressive disorders and ACS were assessed: sociodemographic characteristics, including age, gender, educational status (year), living condition (living alone or not), accommodation type (owned or rented), current occupation (employed or not); depression-related characteristics, including previous and family histories of depression; and cardiovascular risk factors, including previous and family histories of ACS, diagnosed hypertension and diabetes mellitus, hypercholesterolemia (fasting serum total cholesterol level > 200 mg/dL, and/or a history of hyperlipidemia with ongoing treatment), obesity (body mass index [BMI] > 25 kg/m^2), and current smoking status. To determine current cardiac status, the severity of ACS was defined using the Killip classification (Killip and Kimball, 1967), left ventricular ejection fraction (LVEF) was measured using echocardiography, heart rate was assessed using electrocardiography (EKG), and serum cardiac biomarkers, including troponin I and creatine kinase-MB (CK-MB), were quantified.

2.3. The EsDEPACS study: a nested interventional study

Participants in the K-DEPACS study were considered potential participants for the EsDEPACS study and were screened for depressive disorder using the Beck Depression Inventory (BDI; Beck et al., 1961) at 2 weeks after ACS diagnosis as inpatients and every 1 month thereafter for up to 3 months as outpatients. ACS patients with depressive symptoms (BDI > 10) were clinically interviewed by study psychiatrists using the MINI. Of the 378 patients diagnosed with depressive disorder who met the eligibility criteria (detailed in the Supplementary material) and consented to blood sampling, 255 agreed to participate and were randomized to receive either escitalopram (n = 127) or a placebo (n = 128). Assessments were conducted at baseline and at weeks 4, 8, 12, 16, 20, and 24. Patients in the drug group initially received an escitalopram dose of 10 mg/day, but investigators could change the dosage to 5 mg/day or 20 mg/day according to clinical decisions based on drug response and tolerability after the second evaluation. Primary efficacy was measured using the HAMD. Details of the trial and the primary findings regarding the superiority of escitalopram to placebo for the primary depressive and secondary outcomes have been published (Kim et al., 2015c). Of the initial 378 patients, the 123 who refused to join the clinical trial were managed using usual medical interventions for ACS.

2.4. The K-DEPACS 1-year follow-up study

As previously described in the study overview, all participants who completed the baseline assessment were asked to complete a follow-up assessment at 1 year after ACS (mean 12.3 ± 2.4 months) to estimate the incidence and persistence of depression. Of the initial 969 ACS patients, 711 (73%) completed the follow-up assessment, and of these 711 participants, 191 participated in the EsDEPACS trial (96 in the escitalopram group and 95 in the placebo group).

2.5. Statistical analysis

The baseline characteristics, including the sociodemographic and clinical factors of the ACS patients with and without depression, were compared using *t*-tests or Chi-square (χ^2) tests, as appropriate. Factors that were significantly related to depression ($p < 0.05$) were entered as covariates in the adjusted analyses; age was also treated as a covariate due to its well-known relationship with methylation (Florath et al., 2014). For the association analysis between methylation status and prevalent depressive disorder, *t*-tests were used to compare *NR3C1* methylation percentages at three CpG sites and their average values according to depression status. The odd ratios (ORs) for prevalent depressive disorder according to *NR3C1* methylation percentage were calculated using logistic regression models adjusted for baseline covariates. To determine the clinical usefulness of *NR3C1* methylation percentage for the diagnosis of depressive disorder, additional analyses were performed to determine sensitivity, specificity, and cut-off values for the percentage of methylation using receiver operating characteristic curve analysis. For the treatment outcome analysis between methylation status and 24-week remission, *t*-tests were used to compare *NR3C1* methylation percentages between those who did and those who did not achieve remission in the total group, and then separately for the escitalopram and placebo groups. Remission status was defined as a HAMD score ≤7 and was estimated at each follow-up point in the EsDEPACS study. The attainment of remission was considered to have occurred only when remission was sustained to the 24-week study endpoint or to the final assessment point, if earlier.

For the association analysis between methylation status and depressive disorder at 1 year after ACS, *t*-tests were performed to compare *NR3C1* methylation percentages between those with and without incident and persistent depressive disorder. ORs for incident depressive disorder according to *NR3C1* methylation percentages were also calculated using logistic regression models adjusted for baseline covariates, and ORs for persistent depressive disorder according to *NR3C1* methylation percentages were estimated in the same model in conjunction with treatment status (escitalopram, placebo, or medical treatment only). Finally, for the association analysis between methylation status and persistent depressive disorder according to treatment status, *t*-tests were separately performed to compare *NR3C1* methylation percentages between those with and without persistent depressive disorder in the escitalopram, placebo, and medical treatment only groups. To adjust for an overall type I error rate of $p < 0.05$ for multiple comparisons, Bonferroni corrections were conducted (four comparisons: three CpG sites and the average value; 0.05/4 = 0.0125) in the *NR3C1* methylation percentages analyses. All statistical analyses were performed using SPSS 23.0 software (IBM Corp.; Armonk, NY, USA).

3. Results

3.1. Baseline characteristics and recruitment

The overall process for the recruitment and follow-up assessments is detailed in Supplementary Figure 1; a total of 969 participants (84%) from the total K-DEPACS baseline sample (n = 1152) agreed to blood sampling. There were no significant differences between ACS patients who consented to offer blood samples and those who did not in terms of any baseline characteristics (all $p > 0.15$, data not shown). Depressive disorder at baseline (prevalent depressive disorder) was diagnosed in 378 (39%) of the 969 total baseline participants. Of these 378 patients, 255 took part in the EsDEPACS trial (127 in the escitalopram group and 128 in the placebo group). Because 49 participants (19%) left the study after baseline, the remaining 206 participants (104 in the escitalopram group and 102 in the placebo group) constituted the subsample for the treatment outcome analysis.

At 1 year after ACS, 711 (73%) of the 969 baseline participants were successfully followed. Older age and a higher Killip class ($p < 0.05$) were significantly associated with participants being lost to follow-up compared to those who completed the follow-up. At 1 year after ACS, 53 (12%) of the 426 participants without depressive disorder at baseline suffered from incident depressive disorder, and 130 (46%) of 285 participants with depressive disorder at baseline suffered from persistent depressive disorder.

3.2. NR3C1 1 F exon methylation percentage and baseline depressive disorder

Participant characteristics were compared according to depressive disorder status at baseline (Supplementary Table 1). Depressive disorder at baseline was significantly associated with female gender, lower educational level, living alone, rented housing, currently unemployed, a higher HAMD score, the presence of hypertension and diabetes, current smoking, and higher heart rate. The *NR3C1 1 F* exon methylation percentages were also compared according to prevalent depressive disorder status (Table 1). After applying the Bonferroni correction, depressive disorder at baseline was significantly associated with greater *NR3C1 1 F* exon methylation at all CpG sites and a higher average methylation percentage. The logistic regression analysis adjusted for covariates (Table 2) revealed similar results without the Bonferroni correction. All associations between depressive disorder at baseline and *NR3C1* 1 F exon hypermethylation, with the exception of methylation at CpG1, remained significant after application of the Bonferroni correction. Additional tests were performed to evaluate the clinical relevance of *NR3C1 1 F* exon methylation in the diagnosis of depressive disorder at baseline in terms of sensitivity, specificity, and cut-off values for methylation. The optimal methylation percentage cut-off values (with the highest sum of sensitivity and specificity) for the diagnosis of baseline depressive disorder at each CpG site were (data not shown): CpG 1: 16.4 (sensitivity, 63.2; specificity, 62.9), CpG2: 25.7 (sensitivity, 63.5; specificity, 65.7), CpG3: 23.5 (sensitivity, 66.4; specificity, 65.5) and the average value of the CpG sites was 20.1 (sensitivity, 71.7; specificity, 61.4). Although the clinical relevance of methylation percentage was modest, our findings suggest that *NR3C1 1 F* exon methylation is involved in the development of depression in patients with ACS.

Table 1
NR3C1 1 F exon methylation percentages according to the baseline depressive disorder status.

Methylation site	No depressive disorder (N = 591)	Any depressive disorder (N = 378)	p-value*
CpG 1, mean (SD)	15.9 (10.0)	19.3 (9.4)	< **0.001**
CpG 2, mean (SD)	22.9 (14.9)	28.9 (14.6)	< **0.001**
CpG 3, mean (SD)	20.6 (13.3)	26.0 (13.1)	< **0.001**
CpG average, mean (SD)	19.8 (11.9)	24.8 (11.6)	< **0.001**

*p-values for depressive disorder status, using t-tests.
Values in bold type represent statistical significance after Bonferroni correction.

Table 2
Adjusted[a] associations between *NR3C1* 1 F exon methylation percentages and baseline depressive disorder status.

Methylation site	Any depressive disorder	
	Wald	OR (95% CI)
CpG1	4.862	1.04 (1.00-1.06)*
CpG2	8.061	**1.03 (1.01-1.05)**†
CpG3	9.710	**1.04 (1.01-1.06)**†
CpG average	8.818	**1.04 (1.01-1.07)**†

[a]adjusted for age, gender, education, living alone, housing, current employment, score of Hamilton Depression Rating Scale, hypertension, diabetes, current smoking and heart rate.
*p-value < 0.05; † p-value < 0.01.
Values in bold type represent statistical significance after Bonferroni correction.

3.3. NR3C1 1 F exon methylation percentage according to remission and treatment drug in the EsDEPACS trial

There were no significant differences between the escitalopram and placebo groups in any of the baseline characteristics (all p-values > 0.05, data not shown). In the 24-week EsDEPACS trial, the escitalopram group had a higher remission rate (51.9%) than the placebo group (35.3%; $\chi^2 = 6.507$, $p = 0.011$). The *NR3C1 1 F* exon methylation percentages according to remission and treatment drug are given in Table 3. The *NR3C1 1 F* exon methylation percentages were not significantly associated with remission in the total participants (escitalopram and placebo). However, *NR3C1 1 F* exon methylation percentages for the CpG site 1 and the average value were lower among patients in remission compared to patients not in remission in the placebo group after applying the Bonferroni correction, whereas no such difference was observed in the escitalopram group.

3.4. NR3C1 1 F exon methylation percentage according to depressive disorder status at the follow-up assessment

Table 4 shows the comparisons of *NR3C1 1 F* exon methylation percentages according to incident and persistent depressive disorder at 1 year after ACS; neither incident nor persistent depressive disorder at follow-up was significantly associated with *NR3C1 1 F* exon methylation percentages. Table 5 depicts similar results revealed by the logistic regression analysis adjusted for covariates. The associations between *NR3C1 1 F* exon methylation percentages and persistent depressive disorder status based on treatment groups are shown in Table 6. Similar to the associations with follow-up depressive disorder status, there were no significant associations with persistent depressive disorder in the total participants (escitalopram and placebo) or the two groups.

4. Discussion

4.1. Principal findings

Our main finding was a significant association between higher *NR3C1 1 F* exon methylation and the prevalence of depression within 2 weeks after ACS. Moreover, escitalopram treatment was more effective than placebo in reducing depressive disorders regardless of *NR3C1 1 F* exon methylation status. However, there were no significant associations with the incidence and persistence of depression 1 year later.

Table 3
NR3C1 1 F exon methylation percentages according to the remission and treatment drugs in the EsDEPACS trial.

Methylation site	Total			Escitalopram			Placebo		
	No remission (N = 117)	Remission (N = 89)	p-value	No remission (N = 50)	Remission (N = 54)	p-value	No remission (N = 67)	Remission (N = 35)	p-value
CpG1	21.2 (9.9)	18.7 (9.2)	0.059	18.6 (9.4)	19.4 (9.4)	0.665	23.2 (9.9)	17.6 (8.9)	**0.006**
CpG2	32.1 (16.0)	28.9 (16.1)	0.160	28.1 (15.1)	30.3 (16.0)	0.472	35.1 (16.1)	26.8 (16.3)	0.016
CpG3	29.1 (14.0)	25.5 (13.7)	0.068	25.9 (13.5)	26.0 (13.8)	0.976	31.4 (14.0)	24.7 (13.7)	0.023
CpG average	27.5 (12.6)	24.4 (12.1)	0.076	24.2 (11.7)	25.2 (12.1)	0.661	29.9 (12.8)	23.0 (12.1)	**0.010**

p-values using t-tests.
Values are described as mean (SD) of methylation percentages.
Values in bold type represent statistical significance after Bonferroni correction.

The present study is the first to longitudinally assess the association of *NR3C1 1 F* exon methylation with risk and treatment outcomes in depressed patients with ACS. Our finding of a significant association between *NR3C1 1 F* exon hypermethylation and depression within 2 weeks of ACS is consistent with several previous investigations of depression in the general population (Dadds et al., 2015; Roy et al., 2017; Kang et al., 2018). However, investigations of the relationship between hypermethylation of the *NR3C1 1 F* promotor and exon and depression in the general population have yielded inconsistent findings (Chen et al., 2017). These inconsistencies may be explained by the use of varying tissue types, variations in study designs, and differences in the *NR3C1 1 F* regions studied (Chen et al., 2017). The only investigation of treatment outcomes found that higher pretreatment levels of total *NR3C1 1 F* promoter methylation summed across 39 CpG sites, including our CpG1, predicted the treatment response to psychotherapy in patients with post-traumatic stress disorder (PTSD) (Yehuda et al., 2013). However, no previous studies have assessed the association between *NR3C1 1 F* promoter and exon methylation and treatment outcomes in depression. Taken together, the present findings suggest that, at least in the ACS population, patients with *NR3C1 1 F* exon hypermethylation were at increased risk of depression within 2 weeks of ACS and were less likely to achieve remission with placebo. However, further investigation will be necessary to confirm the association between *NR3C1 1 F* exon methylation and depression in ACS and to establish future clinical and research directions.

4.2. NR3C1 methylation and depression risk in ACS

Although the clinical relevance of methylation in terms of sensitivity and specificity was not high, the significant association between *NR3C1 1 F* exon hypermethylation and depression within 2 weeks of ACS suggests that the methylation status of the *NR3C1 1 F* exon may be related to the development of depressive disorder after ACS. The underlying mechanisms may be explained as follows. First, hypermethylation of the *NR3C1 1 F* promoter and exon is associated with atherosclerosis, which is a well-known primary cause of ACS (Zhao et al., 2015) and may decrease GR expression in ACS patients. Hypermethylation of the *NR3C1* gene at the promoter and *1 F* exon regions is related to reductions in GR mRNA levels (Roy et al., 2017; Perroud et al., 2014) and abnormal cortisol responses (Tyrka et al., 2012; Perroud et al., 2014). Thus, it is possible that methylation of the *NR3C1* promoter or *1 F* exon has functional influences and perhaps affects GR expression so as to ultimately dysregulate the stress response in ACS patients. These pathological changes in the HPA axis may result in a reduced ability to cope with stressful situations, such as unexpected ACS, financial and occupational problems, disabilities, or other difficulties that make ACS patients more vulnerable to depression.

Table 5
Adjusted associations between NR3C1 1 F exon methylation percentages and depressive disorder status at follow-up.

Methylation site	Incident depressive disorder[a]		Persistent depressive disorder[b]	
	Wald	OR (95% CI)	Wald	OR (95% CI)
CpG1	0.437	0.99 (0.95-1.03)	0.901	1.01 (0.99-1.04)
CpG2	0.700	1.01 (0.99-1.03)	1.06	1.01 (0.99-1.03)
CpG3	0.567	1.01 (0.99-1.03)	0.377	1.01 (0.99-1.03)
CpG average	0.201	1.01 (0.98-1.03)	0.104	1.01 (0.99-1.03)

[a]adjusted for age, gender, education, living alone, housing, current employment, score of Hamilton Depression Rating Scale, hypertension, diabetes, current smoking and heart rate.
[b]adjusted for the same model as in incident depressive disorder plus treatment status (escitalopram, placebo, and medical treatment only).

Second, the HPA axis also plays an essential role in regulating inflammation. Alterations of the HPA axis that are related to hypermethylation of the *NR3C1 1 F* exon may result in abnormal negative feedback and the failure to produce an anti-inflammatory response (Pariante, 2017). Several studies have shown that altered GR function is related to heightened inflammatory responses in both those with depression and ACS patients (Nikkheslat et al., 2015; Pariante, 2017). Indeed, inflammation plays a crucial role in the pathomechanisms of ACS, and ACS patients tend to exhibit higher clinical markers of inflammation (Hansson, 2005). Thus, excessive inflammation is more likely to occur in ACS patients with than in those without hypermethylation of the *NR3C1 1 F* exon. According to the inflammatory hypothesis for depression, this heightened inflammation may

Table 4
NR3C1 1 F exon methylation percentages according to the depressive disorder status at follow-up.

Methylation site	Incident depressive disorder			Persistent depressive disorder		
	No (N = 373)	Any (N = 53)	p-value	No (N = 155)	Any (N = 130)	p-value
CpG1	16.3 (10.2)	15.7 (10.1)	0.677	19.1 (9.8)	20.5 (9.3)	0.229
CpG2	23.2 (15.3)	24.5 (15.6)	0.578	28.2 (14.9)	30.2 (13.6)	0.243
CpG3	20.9 (13.9)	22.1 (12.3)	0.534	25.8 (13.6)	27.0 (12.6)	0.446
CpG average	20.1 (12.2)	20.8 (11.8)	0.726	24.4 (12.2)	25.9 (11.1)	0.277

p-value using t-tests.
Values are described as mean (SD) of methylation percentages.

Table 6
NR3C1 1 F exon methylation percentages according to treatment and persistent depressive disorder status at one year.

Methylation site	Escitalopram			Placebo			Medical treatment only		
	No depressive disorder (N = 64)	Any depressive disorder (N = 32)	p-value	No depressive disorder (N = 47)	Any depressive disorder (N = 48)	p-value	No depressive disorder (N = 44)	Any depressive disorder (N = 50)	p-value
CpG1	18.2 (9.4)	20.4 (9.0)	0.278	19.5 (10.6)	20.9 (9.2)	0.499	19.9 (9.8)	20.1 (9.6)	0.929
CpG2	27.4 (14.4)	30.7 (16.1)	0.304	29.4 (15.5)	31.0 (14.4)	0.599	28.2 (15.7)	29.2 (11.0)	0.735
CpG3	25.5 (13.2)	27.0 (13.5)	0.600	26.5 (14.8)	28.2 (13.4)	0.556	25.4 (13.0)	25.7 (11.3)	0.889
CpG average	23.7 (11.7)	26.0 (12.3)	0.365	25.1 (12.9)	26.7 (11.8)	0.537	24.5 (12.5)	25.0 (9.8)	0.832

p-value using t-tests.
Values are described as mean (SD) of methylation percentages.

contribute to the development of depression in ACS patients (Dantzer et al., 2008). Because the present study did not examine GR expression (GR mRNA or related proteins) or inflammatory cytokines in the plasma and central nervous system (CNS), further study of the precise mechanisms underlying the relationship between *NR3C1* methylation and depression in patients with ACS is essential.

The present study also found that *NR3C1 1 F* exon hypermethylation was associated with depression within 2 weeks of ACS, but higher levels of methylation were not associated with incident or persistent depression at 1 year after ACS. This suggests that the biological mechanisms that contribute to the development of depression after ACS may be time specific. The effects of *NR3C1 1 F* exon hypermethylation were obvious within 2 weeks of the ACS event when ACS itself acted as a psychological and physiological stressor, resulting in an overwhelmed stress response via the HPA axis. However, this effect may lessen over the first year after ACS as the HPA axis adapts to ACS-related stressors. Furthermore, at 1 year after ACS, psychological and clinical factors such as personality and disability related to occupational functions can contribute to the onset of depression as ACS patients are exposed to new stressors that can cause depression. Thus, the importance of HPA axis contributions to the onset of depression in the year after ACS might be reduced. This hypothesis is supported by previous studies showing that different clinical and psychosocial profiles predict the onset of depression at different time points after ACS (Martens et al., 2008). Moreover, similar time-specific associations between the onset of depression after ACS and tumor necrosis factor-α levels, measured as a representative proinflammatory cytokine, have been reported (Kim et al., 2016). These associations are correlated with HPA-axis function based on previously described biological mechanisms. Additionally, there may be a reverse effect of depression at 1 year after ACS on methylation, in which case these changes may play more important roles in depression at 1 year than at baseline, which contributed to the no significant association between methylation and depression at 1 year after ACS. Our study was restricted to one measurement of NR3C1 methylation at baseline; therefore, further studies that measure methylation status during the follow-up period are needed.

4.3. NR3C1 methylation and depression treatment outcomes

It is noteworthy that the nested escitalopram/placebo-controlled design in the present study provided noticeable findings during the 24-week trial. Although the effects of escitalopram on the remission of depressive symptoms did not depend on the methylation status of the *NR3C1 1 F* exon, only ACS patients with lower levels of *NR3C1* 1 F exon methylation were likely to achieve remission with placebo for 24 weeks. Our study is the first to investigate the associations between remission with antidepressants and *NR3C1 1 F* methylation in depressed patients with and without a physical illness as well as unselected depressive patients. In contrast to our findings, a previous investigation found that higher pretreatment levels of *NR3C1 1 F* promoter methylation predicted the treatment response to psychotherapy in patients with PTSD (Yehuda et al., 2013). This disparity may be explained by differences in treatment modalities (psychotherapy and antidepressants) and the CpG sites investigated. Furthermore, the pathophysiology of PTSD and depression are polar opposites in terms of HPA axis activity (hypersuppression in PTSD vs. non-suppression in depression; Yehuda, 2002). Therefore, it is reasonable to conclude that differences in the direction of the association between *NR3C1 1 F* methylation and treatment response might be due to differences in the site where methylation was measured, the study populations, and treatment modalities. Further investigations will be necessary to elucidate the association between *NR3C1 1 F* exon methylation and treatment outcomes of antidepressants and its directionality using placebo-controlled designs that include ACS patients and individuals from the general population who have depression.

4.4. Strengths of the study

The present study has several strengths. It is the first study to assess the longitudinal association between *NR3C1 1 F* exon methylation and depression after ACS as well as the first to investigate the association between *NR3C1 1 F* exon methylation and treatment outcomes, including remission, utilizing data from a nested double-blind placebo-controlled study. First, a structured diagnostic interview was used to diagnose depressive disorder, and well-validated measurements were used to assess participants' psychiatric and clinical characteristics. Additionally, a comprehensive set of covariates that could potentially affect both depressive disorders after ACS and NR3C1 methylation status were considered in the analyses. Second, depressive disorders and other variables were evaluated at two similar timepoints to reduce heterogeneity caused by different assessment times. Third, the recruitment of participants was conducted consecutively from among all eligible patients who were admitted for recent ACS at the study hospital; a large number of ACS patients were included in the final analyses. This procedure contributed to reducing the probability of selection bias and enhanced the potential generalizability of the findings.

4.5. Limitations

However, several limitations should also be considered when assessing the present findings. First, the present study investigated the methylation level of the *NR3C1 1 F* exon using peripheral blood and not brain tissue. The exact association between *NR3C1 1 F* exon methylation estimations from blood samples and those made from brain tissue, which directly influence mood and behavior, have yet to be established. However, DNA methylation and expression in the peripheral blood and central tissues are likely to be co-regulated (Szyf, 2012), and a recent meta-analysis identified a significant correlation between methylation patterns in the blood and in brain tissue (Tylee et al., 2013). Nonetheless, in this context, careful interpretation of the present findings will be necessary because they may not reflect a direct index of *NR3C1 1 F* exon methylation in the CNS. Second, the methylation statuses of only three CpG sites in a single CpG island of the *NR3C1* gene were measured, and the biological factors representing *NR3C1* gene

expression were not examined. The methylation status of this single island provides limited information about the whole gene, and this may have skewed the associations toward null and obscured true group differences. Unfortunately, this type of measurement error was not accounted for in the observed associations. Moreover, the functional mechanisms underlying the relationship between *NR3C1 1 F* exon methylation and depression in ACS patients remain uncertain due to a lack of investigation of *NR3C1* gene expression. Finally, 73% of the total baseline sample of participants was followed at 1 year after ACS. Attrition was correlated with older age and poorer cardiac status, but not depressive status. It can also be presumed that severe cardiac pathology contributed to the inability of ACS patients to attend follow-up assessments, and this may have obscured the associations of interest. Considering all of these limitations, future replication studies will be necessary to establish the association between *NR3C1 1 F* exon methylation status and the risk and treatment outcomes of depression in ACS patients.

5. Conclusions and implications

In conclusion, ACS patients with higher *NR3C1 1 F* exon methylation levels were more likely to experience depression within 2 weeks after ACS events and less likely to achieve remission with placebo. ACS patients with depressive disorder achieved remission with escitalopram regardless of *NR3C1 1 F* exon methylation status; therefore, those patients with higher *NR3C1 1 F* exon methylation levels should be closely monitored for depression. Moreover, depressed ACS patients should be treated with antidepressants, such as escitalopram, because placebo effect was not evident in patients with high *NR3C1 1 F* exon methylation levels. The present findings suggest that the epigenetic alteration of GRs in the HPA axis plays a role in the onset and treatment outcomes of depression in ACS patients. For clinical purposes, it might be useful to evaluate *NR3C1 1 F* exon methylation status as a potential means of identifying ACS patients at risk for current depression as well as predicting treatment outcomes; this procedure is also noninvasive and convenient. Further research aimed at identifying the underlying mechanisms and consequences of *NR3C1 1 F* exon hypermethylation using larger populations and different ethnic groups will be needed. The present study provides an initial and meaningful contribution regarding the effects of *NR3C1 1 F* exon methylation in the pathophysiology of depression in ACS patients and may serve as a foundation for future research.

Authors' contribution

The study concept, design and interpretation of data were constructed by J-M K, MHJ, and J-SY. Statistical analysis was performed by H-J K. and J-M K. Supervision was conducted by J-M K. The data acquisition and analysis was conducted by H-J K, K-YB, S-W K, I-SS, YJH, YA, H-R K, and M-G S. Drafting of the manuscript was made by Prof. J-M K and H-J K. Critical revision of the manuscript for important intellectual content was conducted by K-YB, S-W K, I-SS, H-R K, M-G S, YJH, YA MHJ, and J-SY. All authors approved the final version of manuscript to be published

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Ethical Statement

The study was performed in accordance with the Declaration of Helsinki.

The study was approved by the institutional review board of Chonnam National University Hospital in Korea.

All procedures were carried out with the adequate understanding and written consent of the subjects.

Conflict of interest

The authors report have no conflict of interest to disclose.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.10.024.

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