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Peripheral blood *GILZ* mRNA levels in depression and following electroconvulsive therapy

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ABSTRACT

Dysregulation of the hypothalamic-pituitary-adrenocortical (HPA)-axis is commonly observed in patients with depression. The delayed feedback system that mediates inhibition of HPA-axis activation is regulated by glucocorticoid receptors (GRs) found in stress-responsive areas of the brain. Glucocorticoid-induced leucine zipper (GILZ) is a key molecule in glucocorticoid biology and is thought to mediate the downstream anti-inflammatory effects of GRs. Previous reports suggest that GILZ levels are altered in the blood and brains of patients with, and animal models of, depression. However, no study has yet investigated the effects of antidepressant treatment on GILZ. Therefore, our aim was to examine peripheral blood *GILZ* mRNA levels in patients with depression (n = 88) compared to age- and sex-matched healthy controls (n = 63), and in patients with depression following treatment with a course of electroconvulsive therapy (ECT). We also assessed the relationship between *GILZ* and mood and clinical outcomes following ECT. *GILZ* mRNA levels were assessed using qRT-PCR. *GILZ* levels were found to be significantly lower in patients with depression compared to controls ($p < 0.002$), and ECT further decreased *GILZ* levels ($p = 0.05$). Both of these results survived adjustment for potential covariates. However, we found no association between *GILZ* and mood scores. Overall, these results suggest that GILZ is involved in the pathophysiology of depression and the peripheral molecular response to ECT.

1. Introduction

Depression is suggested to be precipitated by chronic stress exposure (Khan and Khan, 2017), and a chronic stress response and dysregulation of the hypothalamic-pituitary-adrenocortical (HPA)-axis are commonly observed in patients with depression (Belvederi Murri et al., 2014; Gold et al., 2015; Juruena et al., 2017). Glucocorticoids play an important role in regulating the extent of HPA-axis activation following stressor exposure (Smith and Vale, 2006). In humans, physiological levels of glucocorticoids (GCs) are required for the maintenance of homeostasis; however, chronic exposure to stressors and the concomitant persistent elevation of GC levels can have detrimental consequences, such as downregulation of the immune system, alterations in mood and behavior, and increased susceptibility to disease (Kino, 2018). The delayed feedback system that mediates inhibition of HPA-axis activation is regulated by glucocorticoid receptors (GRs) found in areas in the brain that are responsive to stress (Smith and Vale, 2006). One of the main targets of the GR is glucocorticoid-induced leucine zipper (GILZ), which is thought to mediate the downstream anti-inflammatory effects of the GR by binding to and reducing the availability of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) (Thiagarajah et al., 2014). GILZ, also termed TSC22D3, is a 137 amino acid leucine zipper protein that is a member of the transforming growth factor-β-stimulated clone 22 (TSC-22) family and is expressed in the spleen, lymph nodes, and thymus (D'Adamio et al., 1997), and also in the brain (Yachi et al., 2007). GILZ has six glucocorticoid response elements in its promoter region and so is strongly influenced by GCs and displays circadian expression that is in line with the release of endogenous GCs (Srinivasan and Lahiri, 2017).

Recent studies have implied a role for GILZ in stress and depression (Frodl et al., 2014, 2012; Pandey et al., 2013; Yachi et al., 2007). Following stress exposure in mice, GILZ is significantly increased in the hippocampus (Yachi et al., 2007), a brain area highly implicated in depression (MacQueen and Frodl, 2011). In contrast, GILZ has been shown to be decreased in microglia from mice exposed to social defeat, an animal model of anxiety and depression (Wohleb et al., 2011). Reports have also suggested that peripheral blood *GILZ* mRNA levels are low in patients with depression (Frodl et al., 2014, 2012), and reduced *GILZ* levels have been found in the brains of teenage suicide victims in comparison to matched normal controls (Pandey et al., 2013).

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However, to our knowledge, no study has yet examined the effects of antidepressant treatment on *GILZ* in either animal models of depression or in patients with depression.

Electroconvulsive therapy (ECT) is the most acutely effective treatment for severe, debilitating, and sometimes life-threatening major depressive episodes (UK ECT Review Group, 2003). However, despite being in use for 80 years, its mechanism of action remains incompletely understood. Patients with depression who attain remission following treatment with ECT show resolution of HPA-axis dysregulation (Yuuki et al., 2005). The rodent model equivalent of ECT, electroconvulsive stimulation (ECS), has provided insight into the molecular changes induced by ECT (Duman and Vaidya, 1998). With regard to the GR, ECS has been shown to normalize stress-induced GR changes in the hippocampus (Hageman et al., 2009). However, no study has yet examined the effects of ECS/ECT on GILZ.

Thus, our aim was to carry out an exploratory study to examine peripheral whole blood mRNA levels of *GILZ* in medicated patients with depression compared to healthy controls, and in the patient cohort pre-/post-ECT. We also performed exploratory analyses to assess the relationship between *GILZ* and mood.

2. Material and methods

2.1. Study participants

This study was carried out in accordance with the Declaration of Helsinki (World Medical Association, 2013) and approved by the St Patrick's University Hospital Research Ethics Committee. Written informed consent was provided by all participants. All patients with depression were recruited in St. Patrick's Mental Health Services (http://www.stpatricks.ie/) as part of the EFFECT-Dep (Enhancing the Effectiveness of ECT in Severe Depression; ISRCTN23577151) trial, a real-world, pragmatic, patient- and rater- blinded, non-inferiority trial of patients with major depression that compared the effects of twice-weekly moderate dose bitemporal (1.5× seizure threshold) and high-dose unilateral (6× seizure threshold) ECT (Semkovska et al., 2016). As previously described, ECT was administered twice weekly with hand-held electrodes using methohexital (0.75–1.0 mg/kg) anesthesia and succinylcholine (0.5–1.0 mg/kg) as muscle relaxant (Semkovska et al., 2016). Inclusion criteria: > 18 years old; referred for ECT for treatment of a major depressive episode, as diagnosed by the Structured Clinical Interview for DSM-IV Axis I Disorders (First et al., 1996); pre-treatment Hamilton Depression Rating Scale, 24-item version (HAM-D24) (Beckham and Leber, 1985) score ≥21. Exclusion criteria: ECT in the previous six months; medically unfit for general anesthesia; substance misuse in the previous six months; dementia or other axis I diagnosis. ECT was administered twice weekly. Patients were maintained on their usual medication during the course of treatment.

Fasting blood samples were taken 07:30–09:30 before the first ECT treatment and 1–3 days following the final treatment. Peripheral blood (2.5 mL) was collected into PAXgene© Blood RNA tubes (PreAnalytiX, Qiagen Ltd., Ireland) per manufacturer's guidelines. Samples were stored at room temperature for 24 h, −20 °C for 24 h, and this was followed by storage at −80 °C.

We recruited healthy controls through advertisements in local newspapers and social media. We collected fasting blood samples from controls between 07:30–09:30 on assessment days.

Participants with a chronic immune disorder or neurological disorder were excluded from molecular analyses.

2.2. Clinical assessments

Demographic data and medical/treatment history were documented for all participants. Depression severity and response to ECT was assessed using the HAM-D24. Response was defined as a ≥60% reduction in HAM-D24 and a score ≤16 at end-of-treatment, while remission was defined as a ≥60% reduction in HAM-D24 and a score ≤10 for two weeks following the end-of-treatment.

2.3. Quantitative real-time polymerase chain reaction (qRT-PCR)

We used a PAXgene Blood RNA kit (PreAnalytix) to extract whole blood mRNA and performed reverse transcription using a high capacity cDNA archive kit (Applied Biosystems, UK). Then, mRNA levels were assessed using qRT-PCR on a StepOnePlus™ instrument (Applied Biosystems) using TaqMan® Gene Expression Assays and TaqMan® Fast Advanced Master Mix (Applied Biosystems). Briefly, a master mix was made by combining 5 µL of TaqMan Fast Advanced Master Mix with 0.5 µL of the target primer (GILZ/TSC22D3, Hs00608272_m1) and 0.5 µL of the endogenous control (glyceraldehyde 3-phosphate dehydrogenase; GAPDH, Hs02758991_g) primer. We added 6 µL of the master mix to each well of the PCR plates along with 4 µL cDNA, which was plated in duplicate. The cycling conditions consisted of an initial polymerase activation step of 95 °C for 20 s followed by 40 cycles of 95 °C for 1 s and 60 °C for 20 s. The relative quantification (RQ) levels were calculated using the comparative CT method after normalization to GAPDH. Interplate calibrators were included on each plate to compensate for the variation across qRT-PCR runs, which was accounted for using qBase + software, version 3.1 (Biogazelle, Belgium; www.qbaseplus.com).

2.4. Statistical analysis

SPSS version 21 (IBM Corporation, NY, USA) was used to carry out statistical analyses. We tested all data for normality using Q—Q plots and a Shapiro-Wilk test and the data were then log-transformed where necessary. The baseline clinical and demographic characteristics are presented as means with standard deviations (SD) or number (%) per group where appropriate. Categorical data were analyzed using Chi-square (χ^2) tests.

The RQ data were analyzed using general linear models. We adjusted for potential variance owing to body mass index (BMI; kg/m^2) and smoking, dichotomized into current versus non-smoker, as these have previously been associated with glucocorticoids and/or mood (Kendler et al., 1993; Livingston et al., 2004; Simon et al., 2006; Wirtz et al., 2008). We also adjusted for potential variance associated with age, sex, and presence of diabetes as reports have shown links to glucocorticoids (Crowley et al., 2014; Di Dalmazi et al., 2012; Nicoll et al., 2019; Perlman et al., 2007), and educational attainment, which was significantly different between the groups. For pre-/post-ECT analyses, we included polarity, depression severity at baseline, presence of psychosis, and electrode placement as covariates where appropriate. Correlational analyses were carried out using either Pearson's product-moment correlation coefficient (Pearson's *r*) or Spearman's rank correlation coefficient rho (Spearman's ρ). RQ data are expressed as means with standard error of the mean (SEM). Differences with a p-value ≤ 0.05 were deemed statistically significant. For exploratory subgroup correlation analyses we accounted for multiple comparisons by deeming differences with a p-value ≤ 0.01 to be statistically significant.

3. Results

3.1. Participants

Samples were available for 95 patients with depression enrolled in the EFFECT-Dep trial and 67 healthy controls. Participants with a chronic immune disorder (systemic lupus erythematosus, n = 1) or neurological disorder (stroke: n = 2; Parkinson's disease: n = 3) were excluded from molecular analyses. Data were also unavailable for a number of participants owing to lack of qRT-PCR amplification in the samples. Thus, the mRNA levels of *GILZ* were determined in a total of

Table 1
Demographic and clinical characteristics of participants.

	Depressed Baseline/Pre-ECT (n = 88)	Controls (n = 63)	Statistical test
Age, years	56.1 (14.3)	53.6 (12.7)	t = 1.12, p = 0.27
Sex, No. (%)			
Male	30 (34.1)	22 (34.9)	χ^2 = 0.01, p = 0.92
Female	58 (65.9)	41 (65.1)	
BMI	27 (5.1)	24.7 (3.3)	t = 3.3, p = 0.001
Smokers, No. (%)	36 (40.9)	13 (20.6)	χ^2 = 6.9, p = 0.009
Education, No. (%)			
Primary	14 (15.9)	4 (6.3)	χ^2 = 37.04, p < 0.001
Secondary	51 (58)	11 (17.5)	
Tertiary & Quaternary	23 (26.1)	48 (76.2)	
Bipolar depression, No. (%)	18 (20.5)		
Psychotic depression, No. (%)	21 (23.9)		
Baseline HAM-D24	30.3 (6.2)	3.1 (2.9)	t = 36.1, p < 0.001
Post-ECT HAM-D24	11.2 (8.3)		
Electrode placement, No. (%)			
Unilateral	41 (46.6)		
Bitemporal	47 (53.4)		
Number of ECT sessions	8.2 (2.5)		
Responders, No. (%)	52 (59.1)		
Remitters, No. (%)	44 (50)		
Medications, No. (%) taking			
SSRI	25 (28.4)		
SNRI	43 (48.9)		
TCA	22 (25)		
MAOI	9 (10.2)		
Mirtazapine	30 (34.1)		
Bupropion	4 (4.5)		
Lithium	34 (38.6)		
Sodium Valproate/ Lamotrigine	5 (5.7)		
Antipsychotics	62 (70.5)		
Benzodiazepines	48 (54.5)		
Non-benzodiazepine hypnotics	52 (59.1)		
Pregabalin	5 (5.7)		
Other	18 (20.5)		

Data are presented as means with standard deviations (SD) or number (%) per group where appropriate. Abbreviations: BMI, body mass index; ECT, electroconvulsive therapy; HAM-D24, Hamilton depression rating scale, 24-item version; MAOI, monoamine oxidase inhibitor; SNRI, serotonin-norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant.

88 patients with depression and 63 healthy controls. The groups were balanced for age and sex. The overall demographic and clinical data for both the controls and patients are summarized in Table 1.

3.2. GILZ mRNA levels in medicated patients with depression compared to healthy controls

We first assessed the mRNA levels of *GILZ* in blood from the entire group of medicated patients with depression compared to healthy controls. We initially carried out crude analyses, and subsequently adjusted for potential covariates (age, sex, BMI, smoking, education, presence of diabetes). Levels of *GILZ* were significantly lower in patients with depression compared to controls ($F_{1,149}$ = 9.94, p < 0.002). Adjusting for potential covariates did not alter the result ($F_{1,141}$ = 6.29, p = 0.01; Fig. 1).

We next assessed mRNA levels in patients with unipolar or bipolar depression compared to age- and sex-matched controls. Using crude analyses, we show that levels of *GILZ* were significantly different between groups (p = 0.005). Post-hoc analyses showed that patients with unipolar and bipolar depression had significantly lower levels of *GILZ* in comparison to healthy controls. Adjusting for potential covariates did not alter the results (Table 2).

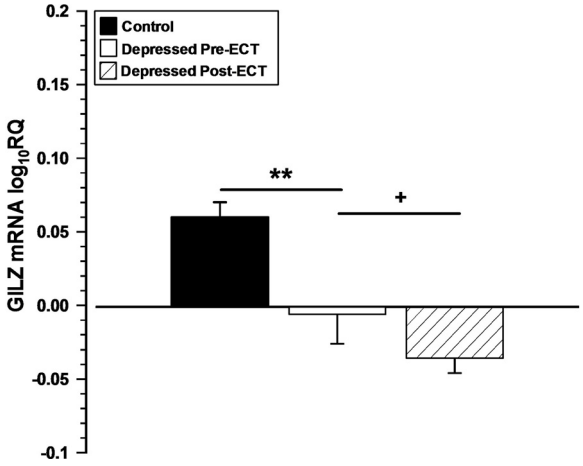

Fig. 1. *GILZ* levels are lower in medicated patients with depression compared to controls and are further reduced by ECT. *GILZ* mRNA levels are lower in blood from medicated patients with depression at baseline (i.e., pre-ECT) compared to healthy controls. *GILZ* mRNA levels were further reduced in patients with depression following treatment with ECT. Data are expressed as unadjusted mean log_{10}RQ ± SEM. **p < 0.001 vs. healthy controls; +p = 0.05 vs. depressed pre-ECT.

We subsequently examined *GILZ* mRNA levels in patients with psychotic or non-psychotic depression compared to age- and sex-matched controls. We show that *GILZ* levels were significantly different between groups (p = 0.009). Post hoc analyses revealed that patients with non-psychotic depression had significantly lower levels of *GILZ* in comparison to healthy controls, but not in comparison to patients with psychotic depression. Adjusting for potential covariates did not alter the results (Table 2).

3.3. GILZ mRNA levels in medicated patients with depression following ECT

Levels of *GILZ* were assessed in blood from medicated patients with depression pre-/post-ECT (Fig. 1). Our initial crude analyses revealed that ECT significantly decreased the mRNA levels of *GILZ* ($F_{1,87}$ = 3.93, p = 0.05), which remained significant after adjusting for potential covariates ($F_{1,76}$ = 4.51, p < 0.04).

We next assessed levels of *GILZ* in patients with unipolar versus bipolar depression pre-/post-ECT (Table 3). Our crude analysis showed that there was no difference in *GILZ* levels in patients with unipolar or bipolar depression pre-/post-ECT. Adjusting for potential covariates resulted in a significant time effect (p = 0.03).

We subsequently assessed *GILZ* levels in patients with psychotic versus non-psychotic depression pre-/post-ECT (Table 3). Our crude analysis showed that there was a significant time effect (p = 0.01) but no significant difference in *GILZ* levels pre-/post-ECT (group × time effect, p = 0.13). Adjusting for potential covariates did not alter the results.

GILZ levels were also examined in patients treated with unilateral versus bitemporal ECT pre-/post-treatment (Table 3). The crude analyses showed that there was a trend towards a significant time effect (p = 0.05) but no significant group or group × time effect (p = 0.78). Adjusting for potential covariates resulted in significant time (p = 0.04) and group (p = 0.03) effects, though no significant group × time interaction (p = 0.78) was found.

Table 2
GILZ mRNA levels in depressed subgroups compared to controls.

	Polarity				
	Control	Unipolar	Bipolar	Statistics	Adjusted Statistics
GILZ[a]	0.0577 (0.02)	0.0015 (0.02)* +	−0.0336 (0.03)*	$F_{2,148} = 5.57$, $p = 0.005$, $\eta_p^2 = 0.07$	$F_{2,142} = 4.37$, $p = 0.01$, $\eta_p^2 = 0.06$
	Psychosis				
	Control	Psychotic	Non-psychotic	Statistics	Adjusted Statistics
GILZ[b]	0.0577 (0.02)	0.0441 (0.03)	−0.0111 (0.02)* +	$F_{2,130} = 4.94$, $p = 0.009$, $\eta_p^2 = 0.07$	$F_{2,142} = 5.78$, $p = 0.004$, $\eta_p^2 = 0.08$

Data are presented as unadjusted mean $\log_{10}$RQ (SEM).
*significantly different to control group in unadjusted analyses.
+ significantly different to control group in adjusted analyses.
[a]Control: n = 63; unipolar: n = 70; bipolar: n = 18.
[b]Control: n = 63; psychotic: n = 21; non-psychotic: n = 67.

3.4. GILZ levels in ECT responders/non-responders and remitters/non-remitters

We first assessed levels of *GILZ* in ECT responders and non-responders (Table 3). We show that there was no difference in levels of *GILZ* in responders or non-responders, or over time. However, we note a trend towards significance in the effect of time on *GILZ* ($p = 0.09$) that became significant after adjusting for potential covariates ($p = 0.04$).

We then examined levels of *GILZ* in ECT remitters and non-remitters (Table 3). We show that there was no difference in the mRNA levels of *GILZ* in ECT remitters or non-remitters, or over time. However, we noted a trend towards significance in the effect of time on *GILZ* ($p = 0.05$), which was strengthened after adjustment for potential covariates ($p = 0.04$).

3.5. Correlations between mood scores and glucocorticoid markers

We initially assessed the relationship between *GILZ* and mood scores in the depressed group as a whole. We found no correlation between baseline *GILZ* and baseline HAM-D24 scores ($\rho = 0.05$, $p = 0.66$), baseline *GILZ* and the change in HAM-D24 scores ($\rho = -0.15$, $p = 0.18$), or the change in *GILZ* and the change in HAM-D24 scores ($\rho = 0.13$, $p = 0.22$).

Our subgroup correlation analyses showed no significant associations between *GILZ* and mood scores (Table 4).

4. Discussion

Our results show that the mRNA levels of *GILZ* were significantly lower in medicated patients with depression compared to healthy controls. Treatment with a course of ECT further decreased the mRNA levels of *GILZ* in the total group of patients with depression, and this survived statistical adjustment for potential covariates. However, we found no associations between *GILZ* and mood scores.

Reports on GILZ in depression have so far been limited. In the social defeat stress animal model of depression, *GILZ* levels were found to be significantly decreased in enriched microglial cultures from socially defeated animals in comparison to those from naïve animals, a finding that was accompanied by an enhanced inflammatory profile, as indicated by an increase in levels of the cytokine interleukin (IL)-1 beta (Wohleb et al., 2011). On the other hand, a study by Yachi et al. (2007) reported that *GILZ* levels are increased in the medial prefrontal cortex (mPFC) and hippocampus (stress-related areas of the brain) of mice exposed to water-immersion restraint stress, and this was shown to be dependent on HPA-axis activation. In humans, reports have suggested that *GILZ* levels are low in patients with depression (Frodl et al., 2014,

Table 3
Analysis of *GILZ* mRNA levels in subgroups of patients with depression pre- and post-ECT.

	Pre-ECT	Post-ECT	Statistics	Adjusted Statistics
Polarity				
Unipolar (n = 70)	0.0015 (0.02)	−0.0276 (0.02)	Time: $F_{1,86} = 2.77$, $p = 0.1$, $\eta_p^2 = 0.03$	Time: $F_{1,76} = 4.51$, $p = 0.03$, $\eta_p^2 = 0.06$
Bipolar (n = 18)	−0.0336 (0.03)	−0.0675 (0.04)	Group: $F_{1,86} = 1.39$, $p = 0.24$, $\eta_p^2 = 0.02$	Group: $F_{1,76} = 0.03$, $p = 0.86$, $\eta_p^2 = 0.0004$
			Group × Time: $F_{1,86} = 0.016$, $p = 0.90$, $\eta_p^2 = 0.0002$	Group × Time: $F_{1,76} = 0.68$, $p = 0.41$, $\eta_p^2 = 0.01$
Psychosis				
Yes (n = 21)	0.0338 (0.02)	−0.0377 (0.02)	Time: $F_{1,86} = 6.30$, $p = 0.01$, $\eta_p^2 = 0.07$	Time: $F_{1,76} = 4.51$, $p = 0.04$, $\eta_p^2 = 0.06$
No (n = 67)	−0.0181 (0.03)	−0.0351 (0.03)	Group: $F_{1,86} = 0.67$, $p = 0.42$, $\eta_p^2 = 0.008$	Group: $F_{1,76} = 0.20$, $p = 0.66$, $\eta_p^2 = 0.003$
			Group × Time: $F_{1,86} = 2.39$, $p = 0.13$, $\eta_p^2 = 0.03$	Group × Time: $F_{1,76} = 1.31$, $p = 0.26$, $\eta_p^2 = 0.02$
Laterality				
Unilateral (n = 41)	0.0164 (0.02)	−0.0142 (0.02)	Time: $F_{1,86} = 3.87$, $p = 0.05$, $\eta_p^2 = 0.04$	Time: $F_{1,76} = 4.51$, $p = 0.04$, $\eta_p^2 = 0.06$
Bitemporal (n = 47)	−0.0249 (0.02)	−0.0545 (0.02)	Group: $F_{1,86} = 2.56$, $p = 0.11$, $\eta_p^2 = 0.03$	Group: $F_{1,76} = 4.64$, $p = 0.03$, $\eta_p^2 = 0.06$
			Group × Time: $F_{1,86} = 0.001$, $p = 0.97$, $\eta_p^2 = 0.00001$	Group × Time: $F_{1,76} = 0.08$, $p = 0.78$, $\eta_p^2 = 0.001$
Responder				
Yes (n = 52)	0.0101 (0.02)	−0.0356 (0.02)	Time: $F_{1,86} = 2.99$, $p = 0.09$, $\eta_p^2 = 0.03$	Time: $F_{1,75} = 4.27$, $p = 0.04$, $\eta_p^2 = 0.05$
No (n = 36)	−0.0284 (0.02)	−0.0359 (0.02)	Group: $F_{1,86} = 0.67$, $p = 0.42$, $\eta_p^2 = 0.008$	Group: $F_{1,75} = 0.28$, $p = 0.60$, $\eta_p^2 = 0.004$
			Group × Time: $F_{1,86} = 1.54$, $p = 0.22$, $\eta_p^2 = 0.02$	Group × Time: $F_{1,75} = 0.09$, $p = 0.77$, $\eta_p^2 = 0.001$
Remitter				
Yes (n = 44)	0.0142 (0.02)	−0.0252 (0.02)	Time: $F_{1,86} = 3.90$, $p = 0.05$, $\eta_p^2 = 0.04$	Time: $F_{1,75} = 4.45$, $p = 0.04$, $\eta_p^2 = 0.06$
No (n = 44)	−0.0255 (0.02)	−0.0462 (0.02)	Group: $F_{1,86} = 1.40$, $p = 0.24$, $\eta_p^2 = 0.02$	Group: $F_{1,75} = 0.003$, $p = 0.96$, $\eta_p^2 = 0.00004$
			Group × Time: $F_{1,86} = 0.38$, $p = 0.54$, $\eta_p^2 = 0.004$	Group × Time: $F_{1,75} = 0.10$, $p = 0.75$, $\eta_p^2 = 0.001$

Data are presented as unadjusted mean $\log_{10}$RQ (SEM). Abbreviation: ECT, electroconvulsive therapy.

Table 4
Correlations between *GILZ* and HAM-D24 scores.

	Baseline *GILZ* & Baseline HAM-D24	Baseline *GILZ* & ΔHAM-D24	Δ*GILZ* & ΔHAM-D24
Polarity			
Unipolar (n = 69)	ρ = 0.03, p = 0.80	ρ = −0.14, p = 0.27	ρ = 0.15, p = 0.22
Bipolar (n = 18)	r = 0.29, p = 0.25	r = −0.34, p = 0.17	ρ = 0.06, p = 0.81
Psychosis			
Yes (n = 21)	r = 0.45, p = 0.04	r = −0.37, p = 0.10	r = 0.27, p = 0.24
No (n = 66)	ρ = -0.13, p = 0.31	ρ = −0.05, p = 0.68	ρ = 0.10, p = 0.44
Laterality			
Unilateral (n = 40)	*not assessed*	*not assessed*	ρ = 0.06, p = 0.72
Bitemporal (n = 47)	*not assessed*	*not assessed*	r = 0.29, p = 0.05
Response			
Yes (n = 52)	ρ = 0.04, p = 0.77	ρ = −0.06, p = 0.70	ρ = 0.09, p = 0.51
No (n = 35)	ρ = 0.03, p = 0.88	r = −0.14, p = 0.43	ρ = −0.001, p = 0.10
Remission			
Yes (n = 44)	ρ = −0.03, p = 0.85	ρ = 0.06, p = 0.72	ρ = −0.04, p = 0.84
No (n = 43)	ρ = 0.14, p = 0.38	ρ = −0.10, p = 0.54	ρ = 0.08, p = 0.62

Abbreviations: HAM-D24Hamilton depression rating scale, 24-item version; *GILZ*glucorticoid-induced leucine zipper; ΔHAM-D24change in Hamilton depression rating scale, 24-item version score; Δ*GILZ* change in *GILZ* levels.

2012; Pandey et al., 2013). In this regard, one report indicated that low peripheral levels of *GILZ* mRNA are associated with smaller hippocampal volumes in patients with major depression (n = 40) compared to healthy controls (n = 43), a finding that was accompanied by increased levels of IL-6 and C-reactive protein (CRP; Frodl et al., 2012). A follow-up study examining the relationship between glucocorticoid inducible genes and hippocampal subfield volumes showed an interaction between low *GILZ* mRNA levels and reduced cornu ammonis (CA)2/3, CA4/dentate gyrus (DG), and subiculum volumes, though these associations did not survive correction for multiple comparisons (Frodl et al., 2014). Furthermore, a study examining *GILZ* mRNA levels in post-mortem brain tissue from teenage suicide victims found decreased levels in the prefrontal cortex and central amygdaloid nucleus compared to controls, though no difference was noted in the hippocampus or medial amygdaloid nucleus (Pandey et al., 2013). Thus, previous reports have suggested that both increased and decreased levels of GILZ are associated with depression and depressive-like behaviour.

As mentioned, GILZ exerts some of its activity by binding to the p65 subunit of the transcription factor NFκB, thereby preventing its translocation to the nucleus (Ayroldi et al., 2001). The target genes of NFκB include those encoding pro-inflammatory cytokines, such as IL-6 and tumor necrosis factor alpha (TNF-α), chemokines, immunoreceptors, and acute phase proteins (Pahl, 1999). Thus, GILZ binding to and inhibiting the activity of NFκB may in turn downregulate levels of IL-6 (Thiagarajah et al., 2014), and other cytokines. Conversely, studies have also shown that cytokines, such as TNF-α and IL-2, can attenuate GILZ levels (Fan and Morand, 2012). Repeated studies have shown increased levels of inflammatory cytokines, including IL-6 and TNF-α, in depression (Kohler et al., 2017). Notably, it has been shown that *IL-6* mRNA levels are repeatedly increased by ECT over the course of treatment, though levels decrease following treatment (Jarventausta et al., 2017). Reports have suggested that cytokines, in particular IL-6, may play a role in neurogenesis (Borsini et al., 2015) and, interestingly, ECS has been shown to increase neurogenesis in the frontal cortex and hippocampal dentate gyrus of rats (Inta et al., 2013; Scott et al., 2000). Moreover, it has been suggested that the acute induction of an inflammatory response in depression may be necessary for the stimulation of neurotrophin release, neurogenesis, and clinical response (Yrondi et al., 2018), and that targeted potentiation of the immune response in depression may be of therapeutic benefit, at least in some subgroups of patients (van Buel et al., 2015).

GILZ can also bind to Raf-1 and Ras, mitogen-activated protein kinase (MAPK) pathway activating molecules, and inhibit their function, thereby modulating downstream MAPK signalling (Fan and Morand, 2012). GILZ-induced inhibition of Raf-1 and Ras activation in turn inhibits phosphoinositide 3-kinase (PI3K), which is involved in cell survival (Fan and Morand, 2012). Importantly, inhibition of PI3K has been shown to increase the risk of a depressive-like phenotype developing in mice (Bandaru et al., 2010). GILZ overexpression has also been shown to inhibit Raf-1 phosphorylation resulting in the inhibition of MEK and extracellular signal-regulated kinase (ERK)1/2 phosphorylation. Notably, Dwivedi et al. (2001) showed that ERK1/2 mRNA levels and activity were significantly decreased in the brains of depressed suicide victims in comparison to controls. Moreover, numerous studies have shown that ERK1/2 is altered in animal models of depression and that its phosphorylation may be involved in the antidepressant mechanism of ECS (Kang et al., 2006).

GILZ is also known to interact with a tandem repeat of CCAAT-enhancer-binding protein (C/EBP) binding sites in the promoter region of peroxisome proliferator-activated receptor gamma (PPAR-γ) and thus inhibit its transcription (Shi et al., 2003). While GCs have been shown to directly activate C/EBP, and in turn PPAR-γ (Shi et al., 2000), GILZ appears to have a direct repressional effect on C/EBP that is in opposition to the effects of GCs (Fan and Morand, 2012). PPAR-γ has been implicated in adult neurogenesis, since it increases cell proliferation, differentiation, and migration (Morales-Garcia et al., 2011). A recent meta-analysis showed that the use of the selective PPAR-γ agonist pioglitazone, either alone or as an adjunctive therapy, induced remission rates in patients with a major depressive episode that were threefold higher than control treatments (Colle et al., 2017). PPAR-γ has also been implicated in the activation of regulatory T-cells, the main role of which is to control inflammation (Ellul et al., 2018). T-cell alterations have been reported in depression, with decreased T-cell activity and a reduced percentage of T-cells noted (Miller, 2010). Importantly, GILZ has roles in both the innate and adaptive immune systems, and has been noted to inhibit T-cell receptor-mediated T-cell activation (Fan and Morand, 2012).

Thus, taking all of the points mentioned above together, the ECT-induced decrease in *GILZ* observed in patients with depression here may lead to beneficial effects by contributing to the increase in inflammatory tone observed following ECT, increased MAPK signalling, and ultimately increased neurotrophin release and neurogenesis and increased T-cell activation (Fig. 2). However, it must be noted that the decrease in GILZ may also contribute to harmful effects. As such, inhibition of GILZ has been shown to contribute to increased

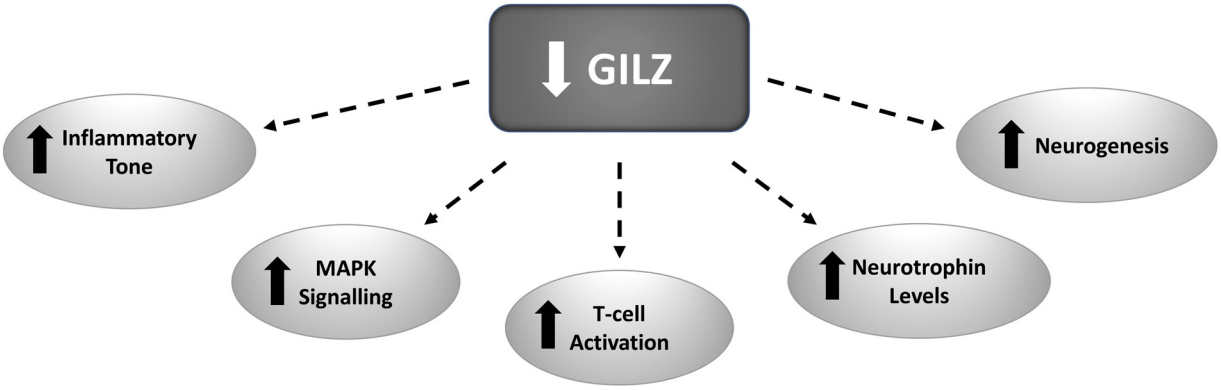

Fig. 2. Potential beneficial effects of low GILZ levels.

susceptibility of neural stem cells to apoptosis since GILZ is known to increase the expression of anti-apoptotic molecules, such as Bcl-2 and Bcl-$_{XL}$ (Srinivasan and Lahiri, 2017). Therefore, further studies are required to fully elucidate the exact role of GILZ in depression and the antidepressant response to ECT.

There are both strengths and limitations to our study. To our knowledge, this is the first study to examine *GILZ* mRNA levels in patients with depression both at baseline in comparison to controls, and also following a course of treatment. However, all of our patients were receiving pharmacotherapy as usual during the course of treatment with ECT. Thus, the low levels of *GILZ* found in patients pre-ECT compared to controls might be an antidepressant effect as opposed to a depression-related effect; however, a previous study suggested that there is no difference in *GILZ* mRNA levels between medicated and unmedicated patients with depression (Frodl et al., 2014). Further studies are required to determine if blood *GILZ* mRNA levels are indeed low in unmedicated patients with depression compared to controls. Other strengths of our study include the relatively large sample numbers that were included in our analyses and the fact that we carried out both crude statistical analyses as well as adjusting for potential covariates. However, a limitation is that the sample numbers included in our subgroup analyses were small in some cases. An additional limitation is that our post-ECT blood samples were taken 1–3 days after completing the ECT course and therefore we cannot comment on whether the reduction in *GILZ* following ECT is an acute effect or if it persists past three days. Thus, time-course studies are required to determine the precise effects of ECT on *GILZ*. A further limitation, as with all blood analyses, is that the changes we found peripherally may not reflect those occurring in the central nervous system.

Overall, our results indicate that GILZ is involved in depression and the peripheral molecular response to ECT.

Declaration of interest

Declan McLoughlin has received a speaker's honorarium from MECTA and an honorarium from Janssen for participating in an esketamine advisory board meeting. KR has no interests to declare. All authors have approved the final article.

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