Unravelling the GSK3β-related genotypic interaction network influencing hippocampal volume in recurrent major depressive disorder

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Objective **Glycogen synthase kinase 3β (GSK3β) has been implicated in mood disorders. We previously reported associations between a *GSK3β* polymorphism and hippocampal volume in major depressive disorder (MDD). We then reported similar associations for a subset of GSK3β-regulated genes. We now investigate an algorithm-derived comprehensive list of genes encoding proteins that directly interact with GSK3β to identify a genotypic network influencing hippocampal volume in MDD.**

Participants and methods **We used discovery ($N = 141$) and replication ($N = 77$) recurrent MDD samples. Our gene list was generated from the NetworKIN database. Hippocampal measures were derived using an optimized Freesurfer protocol. We identified interacting single nucleotide polymorphisms using the machine learning algorithm Random Forest and verified interactions using likelihood ratio tests between nested linear regression models.**

Results **The discovery sample showed multiple two-single nucleotide polymorphism interactions with hippocampal volume. The replication sample showed a replicable interaction (likelihood ratio test: $P = 0.0088$, replication sample; $P = 0.017$, discovery sample; Stouffer's combined $P = 0.0007$) between genes associated previously with endoplasmic reticulum stress, calcium regulation and histone modifications.**

Conclusion **Our results provide genetic evidence supporting associations between hippocampal volume and MDD, which may reflect underlying cellular stress responses. Our study provides evidence of biological mechanisms that should be further explored in the search for disease-modifying therapeutic targets for depression.** *Psychiatr Genet* **28:77–84 Copyright © 2018 The Author(s). Published by Wolters Kluwer Health, Inc.**

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Introduction

Glycogen synthase kinase 3β (GSK3β; OMIM 605004) is a unique pleiotropic protein kinase. It was originally identified for its function involving glycogen synthesis (Embi *et al.*, 1980), but it is now recognized for playing multiple cellular roles in metabolism, transcription, apoptosis, neurogenesis, cell survival, neural differentiation, immune responses, neurotransmitter function and synaptic plasticity (Grimes and Jope, 2001; Kim and Snider, 2011; Beurel *et al.*, 2015; Gao *et al.*, 2016).

GSK3β inhibition has been implicated as a biological mechanism of mood regulation (Li and Jope, 2010), mood stabilizers, antidepressants (Beaulieu, 2012) and treatment-resistant depression (Costemale-Lacoste *et al.*, 2016). Behavioural studies have shown that GSK3β regulates depressive-like behaviours and memory function (Pardo *et al.*, 2016), hippocampal plasticity in maternal separation models (Bian *et al.*, 2015) and models of behavioural despair (Strekalova *et al.*, 2016).

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We previously carried out brain-wide analyses that identified associations between hippocampal volume and a functional *GSK3β* polymorphism (rs6438552) in major depressive disorder (MDD) patients (Inkster *et al.*, 2009). We subsequently reported brain structural associations with a subset of genes that biologically interact with GSK3β (Inkster *et al.*, 2010).

We now examine a comprehensive list of genes that encode proteins that directly interact with GSK3β. We focused on the right hippocampal volume as the phenotype because of previous, region-specific results in the right hippocampus of gene-by-MDD effects of GSK3b-related and several canonical Wnt signalling pathway-related single nucleotide polymorphisms (SNPs) (Inkster *et al.*, 2009, 2010). Independent of this, the same segmentation technique as that applied here has been used widely in large-scale imaging genetic studies on the hippocampus (Hibar *et al.*, 2015, 2017) and in prospective meta-analyses on MDD (Schmaal *et al.*, 2016). Our aim is to identify a *GSK3β*-related genotypic interaction network influencing hippocampal volume in MDD patients using machine learning methods (Nicodemus *et al.*, 2010a, 2010b) applied to discovery and replication samples (Cohen-Woods *et al.*, 2009; Inkster *et al.*, 2009).

Participants and methods

The discovery sample

Major depressive disorder patients

The discovery sample included 145 patients with recurrent MDD described in detail elsewhere (Inkster *et al.*, 2009, 2010). In brief, MDD patients belonged to a cohort of 1022 recurrent MDD patients and 1000 healthy controls (Tozzi *et al.*, 2008). The recruiting hospital obtained approval from the Research Ethical Board. Patients were assessed primarily at the Max Planck Institute of Psychiatry, Munich, Germany. Patients with bipolar disorder, mood incongruent psychotic symptoms, a lifetime history of drug use or diagnosis of drug dependency, depression secondary to alcohol or substance abuse or depression as a result of medical illnesses or use of medications were not included in the study. Age and sex demographics are summarized in Table 1.

Table 1 Demographics for the discovery and replication imaging genetics samples

Samples	Discovery	Replication
N	141	69
Right hippocampal volume [mean (SD)]	4150.8 (387.7)	3950.7 (473.8)*
Female [*n* (%)]	86 (61.0)	48 (69.6)
Age [mean (SD)]	49.2 (13.3)	48.5 (8.1)
ICV (SD)	1 506 774 (160 739)	1 473 184 (235 564)
MRI coil upgrade [*n* (%)]	36 (25.5)	NA

ICV, intracranial volume; NA, not available.
*The mean right hippocampal volume was significantly larger in the discovery sample versus the replication sample (*t*-test, $P = 0.0029$). No other significant differences were observed (all $P > 0.05$, uncorrected). We did not analyse antidepressant medication effects because of missing data across samples and because of the large heterogeneity in medications used.

Structural brain imaging

MRI acquisition

High-resolution T1-weighted MRIs were acquired on a 1.5-T General Electric scanner (Signa, later upgraded to Signa Excite; Waukesha, Wisconsin, USA), inversion recovery prepared spoiled gradient echo recalled with a field-of-view of 22×22 cm^2, a matrix of 256×256, 124 sagittal slices and a resulting voxel size of $(1.2 - 1.4)\ 0.9 \times 0.9$ mm^3 (time to repetition, 10.3 ms; echo time, 3.4 ms; flip angle 20°).

FreeSurfer

We used FreeSurfer (*http://surfer.nmr.mgh.harvard.edu*) to create an optimized protocol to derive right hippocampal volume measures. The *recon-all* command was used to process each T1 image. This process involves the removal of nonbrain tissue using a hybrid watershed/surface deformation procedure, intensity normalization, automated transformation to the Talairach atlas and segmentation of the subcortical grey matter nuclei.

Image quality control

The sample originally included 193 patients. Previous quality control (QC) procedures reduced this number to 145 (detailed in Inkster *et al.*, 2009). In this study, FreeSurfer images were inspected visually to ensure accuracy of registration and segmentation procedures. The sample was reduced to 141 [three patients were excluded with ± 3 SD and one with a missing value for the covariate, intracranial volume (ICV)]. The QC measures that we applied were consistent across the discovery and replication samples. We did, however, observe a difference in the percentage of participants lost to QC across the two cohorts (~26% in the discovery sample vs. 10% in the replication sample). This could be related to site-specific participant-related issues (i.e. increased head motion at this site) or differences in the scanner data collection process or software packages used, etc.

The replication sample

Major depressive disorder patients

The replication sample included 77 recurrent MDD patients recruited at the Institute of Psychiatry, Psychology and Neuroscience, King's College London, UK. Patients had previously participated in genetic association studies (Uher *et al.*, 2008; Cohen-Woods *et al.*, 2009) and imaging genetics studies (Cole *et al.*, 2011, 2013). The Bexley and Greenwich NHS Research Ethics Committee approved this study. Patients had experienced two or more depressive episodes of at least moderate severity, separated by at least 2 months of remission. The diagnosis was made using the Schedules for Clinical Assessment in Neuropsychiatry interview (Wing *et al.*, 1990) according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed. (DSM-IV) criteria. Exclusions were made if the patient, or a first-degree relative, ever fulfilled the

criteria for mania, hypomania, schizophrenia or mood incongruent psychosis, had a diagnosis of any neurological disorder or other condition known to affect brain structure or function. Other exclusion criteria included a lifetime diagnosis of alcohol or substance abuse, depression only secondary to medical illness or medication, a diagnosis of mania or psychosis in first-degree or second-degree relatives or a contraindication to MRI. Age and sex demographic details are described in Table 1.

Structural brain imaging

MRI acquisition, freesurfer and image quality control

Magnetization-Prepared Rapid Gradient Echo T1-weighted scans were collected at the Institute of Psychiatry, King's College London, on a 1.5 T Signa HDx system (General Electric, Boston, Massachusetts, USA). Acquisition parameters were as follows: echo time = 3.8 ms, repetition time = 8.59 ms, flip angle = 8°, field-of-view = 24 cm × 24 cm, slice thickness = 1.2 mm, number of slices = 180 and image matrix = 256 × 256. We used the same FreeSurfer protocol as that described in the discovery sample.

GSK3β network gene list

Our *GSK3β* gene network list (Supplementary Table S1, Supplemental digital content 1, *http://links.lww.com/PG/A206*) was derived using the NetworKIN algorithm from Linding *et al.* (2007); *http://networkin.lindinglab.org*, which integrates consensus substrate motifs (NetPhorest) with context modelling (STRING) to improve the prediction of cellular kinase–substrate relations (Linding *et al.*, 2007). Gene boundaries were as given in NCBI Gene and dbSNP.

Genetic data

The discovery sample

Whole-genome scan genotypes were obtained following the QC procedures described elsewhere (Tozzi *et al.*, 2008; Muglia *et al.*, 2010). In brief, genotypes were obtained using two-channel signal intensity data, corresponding to the two alleles at each SNP that were evaluated using Beadstudio 3.1 (Illumina Inc., San Diego, California, USA). The initial genotype calls were generated using the cluster file. The whole-genome association analysis of the full sample of patients and controls produced a genomic control of $\lambda = 1002$ (Muglia *et al.*, 2010). The data were imputed as part of the Psychiatric Genomics Consortium MDD genome-wide association study to HapMap3 reference sequence using the utah residents with northern and western European ancestry from the CEPH collection and Toscani in Italia populations. Of the 271 genes in the network, we removed nonautosomal genes ($N = 9$) and genes that contained no SNPs ($N = 10$) (Supplementary Table 1, Supplemental digital content 1, *http://links.lww.com/PG/A206*). Gene boundaries were as given in NCBI Gene and dbSNP. A total of 8846 SNPs were available in the 252 genes. Hard-called genotypes from dosage data were used in the interaction analyses, with a dosage hard call threshold of 0.8 using PLINK v1.0.7. Missing genotypes (missingness range per individual = 1.3–3.5%) were imputed using median imputation as the Random Forest (RF) algorithm does not handle missing values. The Hardy–Weinberg Equilibrium threshold P value was set to 0.001; none were removed. Before analysis with RF, SNPs were linkage disequilibrium (LD)-pruned ($r^2 = 0.25$) as strongly correlated predictors can influence the results of RF (Nicodemus and Malley, 2009; Nicodemus *et al.*, 2010c; Nicodemus, 2011), leaving a total of 1155 SNPs for analysis.

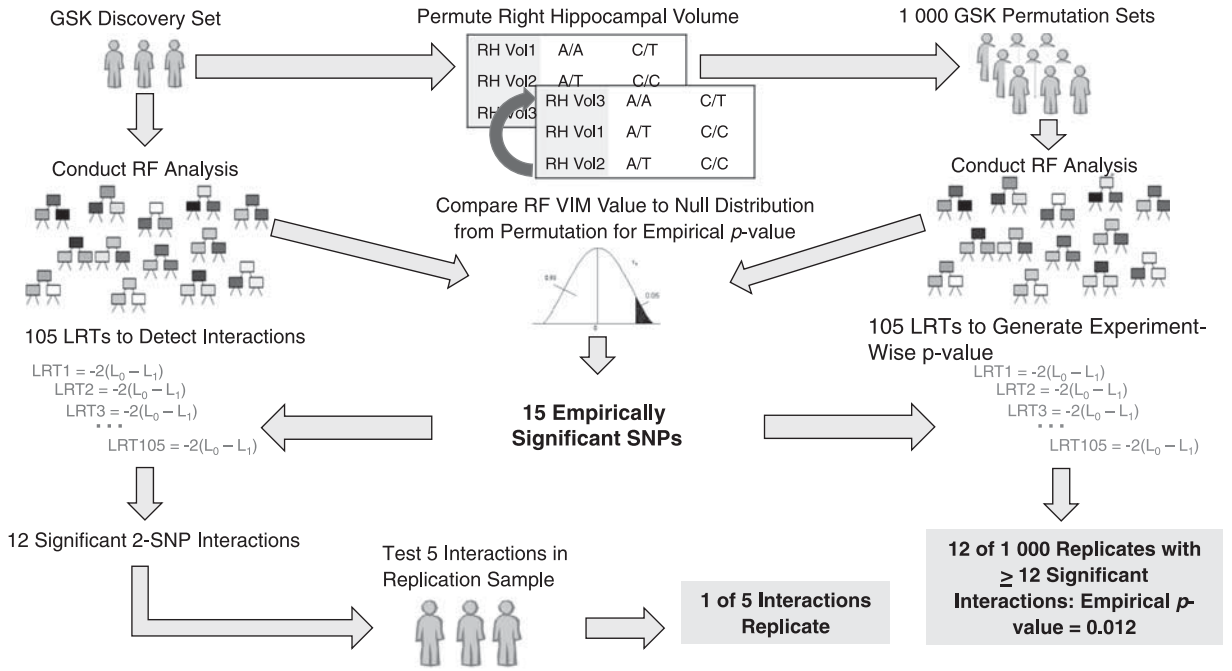
The replication sample

Genotypes were derived from genome-wide microarray data described elsewhere (Lewis *et al.*, 2010). DNA samples were genotyped using the Illumina HumanHap610-Quad BeadChips (Illumina Inc.) by the Centre National de Genotypage (Evry, France). Patients were excluded on the basis of missingness per individual greater than 1% or abnormal heterozygosity. A single patient was excluded if pairs of patients showed greater than second-degree relatedness. SNPs were excluded if they showed a departure from Hardy–Weinberg Equilibrium with a P value less than 0.00001. Principal component analysis was carried out using EIGENSTRAT (Price *et al.*, 2006) after QC procedures. Imputation was performed after using LiftOver to map SNPs from hg18 to hg19 coordinates and then the Genotype Harmonizer (Deelin *et al.*, 2014) was used to prepare the genotypes for imputation through alignment to the Haplotype Reference Consortium (McCarthy *et al.*, 2016). Phasing and imputation were completed on the Michigan imputation server (Das *et al.*, 2016) using the Haplotype Reference Consortium reference panel, version r1.1, phasing using Eagle v.2.3 (Loh *et al.*, 2016) with the EUR population. Of the 77 individuals, the following were excluded before analysis: two after failing imaging QC, one with inconsistent sex-versus-genotype data reported, one with non-European ancestry and four after failing genotyping or imputation QC, leaving 69 for analysis.

Statistical analyses

Initial analysis of the discovery sample used standard single SNP linear regression models on right hippocampal volume, controlling for age, sex, ICV and head coil upgrade. The discovery sample analysis was carried out using the machine learning algorithm RF (Breiman, 2001), which is designed for high-dimensional data sets, and its variable importance measure, used here, captures the main effects of single predictors as well as complex interactions. This method has successfully identified validated epistasis in the context of IQ in psychosis and in schizophrenia case–control genomics data (Nicodemus *et al.*, 2010a, 2010b). To control for the effects of sex, age, ICV and imaging head coil upgrade in the RF analysis, we regressed these variables of noninterest out on both

Fig. 1



Schema of Random Forest analysis. GSK, glycogen synthase kinase; LRT, likelihood ratio test; RF, Random Forest; SNP, single nucleotide polymorphism.

sides of the equation (right hippocampal volume and SNPs) and used the residuals as input as described previously (Zhao *et al.*, 2012). For the RF analysis, the number of variables selected at each split of the tree (*mtry*) was set to 300 and the number of trees constructed per forest was 1000 (Fig. 1). We used the permutation-based variable importance measure as a measure of association between SNPs and outcome. To obtain stable estimates of the variable importance measures, we re-ran RF on the same data 1000 times, changing the random number seed each time, and used the median of these variable importance values as the final set of variable importance measures (Nicodemus and Malley, 2009). We re-ran the RF algorithm on 1000 sets of data in the discovery sample where the outcome had been permuted randomly to obtain a null distribution of variable importance measures for each SNP (Nicodemus, 2011) to calculate an empirical *P* value. The empirical *P* value associated with RF variable importance measures was used to determine which SNPs would be tested for a two-way interaction using likelihood ratio tests (LRT) between nested linear regression models:

$$\begin{aligned}\text{Full model}: \text{Right\_hippocampal\_volume} &\sim \beta_1\,\text{age}+\beta_2\,\text{sex}+\beta_3\,\text{ICV}+\beta_4\\ &\text{Head\_coil\_upgrade}+\beta_5\,\text{SNP}_\text{i}\\ &+\beta_6\,\text{SNP}_\text{j}+\beta_7\,\text{SNP}_\text{i}\times\text{SNP}_\text{j}.\end{aligned}$$

$$\begin{aligned}\text{Reduced model}: \text{Right\_hippocampal\_volume} &\sim \beta_1\,\text{age}+\beta_2\,\text{sex}+\beta_3\,\text{ICV}+\beta_4\\ &\text{Head\_coil\_upgrade}+\beta_5\,\text{SNP}_\text{i}+\beta_6\,\text{SNP}_\text{j}.\end{aligned}$$

The 1000 null replicates were used to calculate an empirical experiment-wise *P* value for the number of significant LRTs out of the 300 possible two-way interactions from the reduced list provided by RF. Replication was attempted only for those models showing *P* values less than 0.05 uncorrected. For the replication sample analyses, linear regression models were used and LRTs between nested models tested the significance of the interaction, just as we had done above for the discovery sample. The replication sample model included sex, age, 10 principal components to control for population stratification and ICV as covariates. Only 19 SNPs from the two-SNP interactions were available; of these, five two-SNP interaction pairs were found where both SNPs were available for analysis (rs12469994–rs2291862, rs12469994–rs939626, rs2291862–rs1052751, rs11780700–rs1052751 and rs939626–rs4387877).

Expression quantitative trait loci analysis

For all SNPs identified to be associated significantly with hippocampal volume in the RF analysis, we carried out an expression quantitative trait loci analysis using the BRAINEAC database (Ramasamy *et al.*, 2014). The BRAINEAC database has a larger number of brain tissue

samples than Genotype-Tissue Expression (Carithers and Moore, 2015); in addition, BRAINEAC individuals were confirmed to be of European descent, like our sample, and also neuropathologically normal. In contrast, Genotype-Tissue Expression individuals include those who have neurological causes of death.

Healthy control sample analysis

Replicated interactions in the MDD samples were tested for interaction with 147 healthy control participants available from the discovery sample (Inkster *et al.*, 2009) using the same model and QC protocol. Overall, 153 healthy control participants were available originally for analysis; three did not pass imaging QC, two had missing covariate or genotype values and one was excluded because their right hippocampal volume was greater than 3SDs from the mean of controls.

Results

Demographics

The mean right hippocampal volume was significantly larger in the discovery sample versus the replication sample (*t*-test, $P=0.0029$). No other significant differences were observed (all $P>0.05$, uncorrected). We did not analyse antidepressant medication effects because of missing data across samples and because of a large heterogeneity in the medications used.

Discovery sample

No single SNP was associated significantly with right hippocampal volume in the discovery sample (Supplementary Table S2, Supplemental digital content 2, *http://links.lww.com/PG/A207*). The most strongly associated single SNP was rs7364220, an intronic variant in the gene *PPARA* ($P=6.36E-05$; Bonferroni threshold = 5.654E − 06). RF analysis showed 15 SNPs with empirical *P* values less than 0.05 (Table 2) that were then subjected to all possible two-way interaction modelling using linear regression, controlling for age, sex, head coil upgrade and ICV, resulting in 105 tests in the discovery sample. Ten of the 15 of the RF-significant SNPs also had single SNP *P* values less than 0.05, uncorrected, and all except one SNP were found to participate in one to three two-SNP interactions using LRTs between nested models (Supplementary Table S3, Supplemental digital content 3, *http://links.lww.com/PG/A208*). Histone deacetylase 4 (*HDAC4*) had two SNPs participating in interactions. Although no two-SNP interaction LRT *P* value passed correction for multiple testing (Bonferroni-corrected critical value = 0.00048), given 105 two-SNP interaction models, the expected number of interactions with a LRT *P* value less than 0.05 is 5.25; we observed 12 using our SNPs as identified as significant with RF. This excess of LRT *P* values less than 0.05 was not because of SNPs in LD as SNPs were LD-pruned before RF analysis. To obtain an experiment-wise null distribution of the number of interactions with an LRT *P* value less than 0.05, we re-ran all 105 two-SNP interactions on 1000 null replicates where the phenotype had been permuted without replacement using the same model as in the analysis of the observed data. Twelve of the 1000 replicates showed at least 12 interactions with an LRT *P* value less than 0.05 (empirical experiment-wise $P=0.012$).

Table 2 Random Forest-identified empirically significant single nucleotide polymorphisms associated with right hippocampal volume in major depressive disorder patients

SNPs	Single SNP *P* value	RF empirical *P* value	Two-SNP interactions (*n*)	Gene	Function
rs4844550	7.53E−05	0.044	2	MAPKAPK2	Upstream variant
rs3791424	*0.0081*	0.021	1	HDAC4	Intron
rs12469994	*0.003*	0.014	2	HDAC4	Intron
rs2291862	*0.012*	0.02	2	ITPR1	Synonymous
rs13083813	0.14	0.017	2	TGFBR2	Intron
rs3798290	*0.058*	0.016	1	FHL5	Intron
rs4720279	*0.0052*	0.012	1	AMPH	Intron
rs2058502	0.0044	0.003	2	EGFR	Intron
rs11780700	*0.034*	0.034	2	C8orf44, SGK3	Intron
rs11014511	0.0098	0.049	1	CACNB2	Intron
rs939626	0.089	0.01	2	IGF1R	Intron
rs1052751	0.21	0.004	3	PLD2	Synonymous
rs11654719	0.0052	<0.001	1	PRKCA	Intron
rs2279103	*0.18*	0.031	0	CTDP1	Missense
rs4387877	*7.53E−05*	0.002	2	PLCB1	Intron

Single SNP *P* values in italics indicate a negative association.
RF, Random Forest; SNP, single nucleotide polymorphism.

Replication sample

Five interaction models were taken forward for testing in the replication sample. One interaction model was replicated showing the same direction of effect. The model included *HDAC4* rs12469994 and *ITPR1* rs2291862, the most significant interaction in the original discovery sample. Individuals who carried more copies of minor alleles at both SNPs showed a significant decrease in hippocampal volume in both the discovery and the replication samples (replication sample LRT $P=0.0088$, $\Delta r^2=0.027$; and discovery sample LRT $P=0.017$, $\Delta r^2=0.072$). Combining *P* values across the two independent samples using Stouffer's *Z* trend (which takes into account the individual *P* values, the sample size and the direction of effect) led to a combined *P* value of 0.0007 for the *HDAC4–ITPR1* interaction.

Healthy control sample analysis

The replicated interaction in the MDD samples between *HDAC4* and *ITPR1* was tested for interaction using 147 healthy control participants available from the discovery sample (Inkster *et al.*, 2009) using the same model and QC protocol (see the Participants and Methods section for details). The LRT between nested models, testing for interaction effects, was not significant ($P=0.77$). In addition, the main effects for both SNPs were also not significant in the full model or in the model with main effects and no interaction term (all $P>0.83$).

Expression quantitative trait loci analysis

Our analysis showed a significant association between the SNP identified in our study, rs2291862, and *ITPR1*

hippocampal expression ($P=0.0045$) as well as the SNP, rs12469994, associated with *HDAC4* hippocampal gene expression ($P=0.017$). We also observed that rs12469994 was related to ASB1 gene expression; however, it is unclear as to how this relates to our findings. A full set of results can be found in Supplementary Table 4 (Supplemental digital content 4, *http://links.lww.com/PG/A209*).

Discussion

Our study aimed to identify a *GSK3β*-related genotypic interaction network influencing hippocampal volume in MDD using a comprehensive list of known proteins that bind to GSK3β. Using two independent imaging genetics recurrent MDD data sets, we confirmed a significant genotypic interaction (with hippocampal volume) in genes linked to endoplasmic reticulum (ER) stress, calcium regulation and histone deacetylase modifications.

Our findings are important for several reasons. This is the first psychiatric imaging genetics study to systematically examine a comprehensive list of genes with direct biological GSK3β interactions. It is therefore the first examination of putative genotypic combinations amongst this network. We used a machine learning algorithm in the discovery sample that explicitly models both genetic main effects and interactions through creating recursively partitioned trees. Given that these genes interact physically in this biological network, we hypothesized that an epistatic effect may be present. We did not observe any single SNP effects that were significant after multiple testing, whereas we discovered and replicated a two-SNP interaction between *HDAC4* and *ITPR1* that was associated with decreased hippocampal volume among MDD patients carrying putative 'risk' alleles at both SNPs.

Inositol 1,4,5-triphosphate receptor, type 1 (*ITPR1*; OMIM 147265), is a calcium channel that regulates the release of calcium from the ER (Yamada *et al.*, 1994). The ER contains the largest reservoir of calcium in the cell. It is also responsible for the correct folding of proteins before their delivery into the cytoplasm. When the ER system is stressed, a large amount of calcium is released into the cytoplasm, which can lead to apoptosis. Our identification of *ITPR1* can be interpreted using the framework proposed by Gold *et al.* (2013), suggesting that impaired ER stress responses play a role in depression. Our study adds to the literature of genetic associations with ER stress and mood disorders (Kakiuchi *et al.*, 2003, 2007; Grunebaum *et al.*, 2009; Hayashi *et al.*, 2009; Nevell *et al.*, 2014), in particular, the discovery of an *ITPR1* gene variant that was amongst the most significant SNPs in an MDD genome-wide association study meta-analysis (Muglia *et al.*, 2010).

The unfolded protein response (UPR) system is a cellular defensive mechanism activated in response to ER-related protein misfolding. Timberlake and Dwivedi (2016) investigated the role of the UPR system in depression. The authors reported hippocampal upregulation of two critical UPR markers (GRP78 and GRP94) in rats with learned helplessness. GRP78 and GRP94 are highly involved in apoptosis and inflammation. Evidence has implicated these processes in the aetiology of depression (Jope *et al.*, 2016; Mechawar and Savitz, 2016). Additional evidence showed that mood disorders may involve mechanisms related to ITPR, ER stress and GSK3β signalling, albeit using an endothelial cell degeneration model in prefrontal cortical tissue (Kurauchi *et al.*, 2016). Therefore, maintaining an efficient ER stress response and UPR system may play a role in the treatment of mood disorders.

HDAC4 was another gene identified in our study. HDAC4 regulates gene transcription by interacting with transcription factors, signal transduction molecules and HDAC3 to carry out many cellular functions, such as proliferation, differentiation, neuronal survival and synaptic plasticity (Wu *et al.*, 2016). Hobara *et al.* (2010) reported increased HDAC4 mRNA expression in patients with unipolar and bipolar depression. In addition, Sarkar *et al.* (2014) reported that viral-mediated hippocampal HDAC4 overexpression was associated with a significant increase in depression-like behaviour in a preclinical model.

Our findings may be relevant for developing future hypotheses involving cognitive impairments in MDD, especially given previous evidence implicating *GSK3β* in cognition (O'Leary and Nolan, 2015). For example, the ER stress inhibitor, tauroursodeoxycholic acid, may alleviate dysfunction of cognition (Cai *et al.*, 2015) and preclinical evidence has shown that ER stress-induced hippocampal apoptosis and cognitive impairments were inhibited by pretreatment with the ER stress inhibitor, salubrinal (Zhang *et al.*, 2014; Ge *et al.*, 2015). Salubrinal has been shown to exert neuroprotective effects (Rubovitch *et al.*, 2015), but it has not been tested in human clinical trials. HDAC4 may also play a role in cognitive function (Wu *et al.*, 2016). The gold standard and commonly used mood stabilizers for the treatment of bipolar disorder, lithium and divalproate, have been implicated to exert HDAC and GSK3β inhibitory effects. A study by Sharma and Taliyan (2015) showed that cognitive impairments in rats treated with a low-dose combination treatment of lithium and divalproex showed improved spatial learning and memory.

Our findings have direct biological relevance to other molecular targets implicated previously in mood disorder pathophysiology, including the noncoding microRNA precursor, miR-124 (Roy *et al.*, 2017). Higuchi *et al.* (2016) found that miR-124-mediated regulation of HDAC4 and GSK3β hippocampal expression may have implications for chronic stress and depression. miR-124 has been identified as a biological mechanism underlying the effects of erythropoietin treatment, which may be relevant to mood disorder treatment, cognitive improvements and increased hippocampal volume (Inkster *et al.*, 2018). Another related molecular target is peroxisome proliferator-activated receptor γ (PPARG), supported by evidence that PPARG activation

improves depressive-like behaviours (Gold *et al.*, 2013), plays a protective role against ER stress (Gold *et al.*, 2013) and PPARG prosurvival activity is inhibited by HDAC4 activation (Yang *et al.*, 2011).

Our study has several limitations. Although this work suggests a potential genetic network associated with brain changes in depression with *GSK3β*, it does not differentiate between whether these MDD-specific genotype-dependent brain structural associations are related to the pathogenesis of MDD or occur as a consequence of disease expression. As there is evidence showing that neuroplastic or neurodegenerative processes cause structural brain changes with depression, stress and pharmacotherapy, this impact of stress, depression and medications may influence hippocampal morphology. We did not test whether these structural changes are specific to major depression. We restricted our analysis to the right hippocampus on the basis of our previous findings (Inkster *et al.*, 2009); however, future work could examine both hippocampi and relevant regions in temporal and prefrontal cortices. Both of the samples used in this study involved recurrent MDD patients. Therefore, we could not consider hypotheses related to early-onset MDD or first-episode MDD to delineate disease processes across time; for example, in first-episode MDD patients, the literature suggests that there are no hippocampal volume deficits (Schmaal *et al.*, 2016) and so it remains unknown how or whether our identified biological mechanisms would be involved. We did not have access to high-quality data related to age of onset or illness duration consistently across both samples; however, the literature suggests that its correlation with age is quite strong and so it is unlikely that it would have impacted on our results significantly. Nonetheless, we accept that this is a limitation of our paper. There are neuroimaging methodological differences for generating hippocampal volume measures between our current study (i.e. FreeSurfer software was used to measure the entire volume of the right hippocampus) that differ from our previous study (i.e. a SPM software-based brain-wide voxel-wise cluster-based method was used, which identified a cluster within the right hippocampus; Inkster *et al.*, 2009). Furthermore, we used statistical methodologies in this study that differed from those of our previous work (Inkster *et al.*, 2009, 2010), which adds complexities to interpretation of these findings collectively.

Conclusion

Our study provides genetic evidence supporting associations between hippocampal volume and recurrent MDD, suggesting that ER stress inhibition and HDAC4 modifications should be explored in the search for disease-modifying therapeutic targets for depression. They also encourage additional drug classes and medications to be considered, and pharmacogenetic studies and clinical trials should be designed to assist with translating these scientific findings into clinical practice.

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Conflicts of interest

B.I., T.N., P.M. and P.M.M. were employees of GlaxoSmithKline when the original data were collected. For the remaining authors, there are no conflicts of interest.

References

Beaulieu JM (2012). A role for Akt and glycogen synthase kinase-3 as integrators of dopamine and serotonin neurotransmission in mental health. *J Psychiatry Neurosci* **37**:7–16.

Beurel E, Grieco SF, Jope RS (2015). Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. *Pharmacol Ther* **148**:114–131.

Bian Y, Yang L, Wang Z, Wang Q, Zeng L, Xu G (2015). Repeated three-hour maternal separation induces depression-like behavior and affects the expression of hippocampal plasticity-related proteins in C57BL/6N mice. *Neural Plast* **2015**:627837.

Breiman L (2001). Random forests. *Mach Learn* **45**:5–32.

Cai F, Liu J, Li C, Wang J (2015). Critical role of endoplasmic reticulum stress in cognitive impairment induced by microcystin-LR. *Int J Mol Sci* **16**:28077–28086.

Carithers LJ, Moore HM (2015). The Genotype-Tissue Expression (GTEx) Project. *Biopreservation and Biobanking* **13**:307–308.

Cohen-Woods S, Gaysina D, Craddock N, Farmer A, Gray J, Gunasinghe C, *et al.* (2009). Depression Case Control (DeCC) Study fails to support involvement of the muscarinic acetylcholine receptor M2 (*CHRM2*) gene in recurrent major depressive disorder. *Human Molecular Genetics* **18**:1504–1509.

Cole J, Weinberger DR, Mattay VS, Cheng X, Toga AW, Thompson PM, Fu CHY (2011). No effect of 5HTTLPR or BDNF Val66Met polymorphism on hippocampal morphology in major depression. *Genes Brain Behav* **10**:756–764.

Cole JH, Boyle CP, Simmons A, Cohen-Woods S, Rivera M, McGuffin P, *et al.* (2013). Body mass index, but not FTO genotype or major depressive disorder, influences brain structure. *Neuroscience* **252**:109–117.

Costemale-Lacoste JF, Guilloux JP, Gaillard R (2016). The role of GSK-3 in treatment-resistant depression and links with the pharmacological effects of lithium and ketamine: A review of the literature. *Encephale* **42**:156–164.

Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, *et al.* (2016). Next-generation genotype imputation service and methods. *Nat Genet* **48**: 1284–1287.

Deelin P, Bonder MJ, van der Velde KJ, Westra HJ, Winder E, Hendriksen D, *et al.* (2014). Genotype harmonizer: automatic strand alignment and format conversion for genotype data integration. *BMC Res Notes* **7**:901–902.

Embi N, Rylatt DB, Cohen P (1980). Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. *Eur J Biochem* **107**:519–527.

Gao L, Zhao M, Ye W, Huang J, Chu J, Yan S, *et al.* (2016). Inhibition of glycogen synthase kinase-3 (GSK3) promotes the neural differentiation of full-term amniotic fluid-derived stem cells towards neural progenitor cells. *Tissue Cell* **48**:312–320.

Ge HW, Hu WW, Ma LL, Kong FJ (2015). Endoplasmic reticulum stress pathway mediates isoflurane-induced neuroapoptosis and cognitive impairments in aged rats. *Physiol Behav* **151**:16–23.

Gold PW, Licinio J, Pavlatou MG (2013). Pathological parainflammation and endoplasmic reticulum stress in depression: potential translational targets through the CNS insulin, klotho and PPARG systems. *Mol Psychiatry* **18**:154–165.

Grimes CA, Jope RS (2001). The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. *Prog Neurobiol* **65**:391–426.

Grunebaum MF, Galfalvy HC, Huang YY, Cooper TB, Burke AK, Agnello M, *et al.* (2009). Association of X-box binding protein 1 (XBP1) genotype with morning cortisol and 1-year clinical course after a major depressive episode. *Int J Neuropsychopharmacol* **12**:281–283.

Hayashi A, Kasahara T, Kametani M, Toyota T, Yoshikawa T, Kato T (2009). Aberrant endoplasmic reticulum stress response in lymphoblastoid cells from patients with bipolar disorder. *Int J Neuropsychopharmacol* **12**:33–43.

Hibar DP, Stein JL, Renteria ME, Arias-Vasquez A, Desrivières S, Jahanshad N, *et al.* (2015). Common genetic variants influence human subcortical brain structures. *Nature* **520**:224–229.

Hibar DP, Adams HHH, Jahanshad N, Chauhan G, Stein JL, Hofer E, *et al.* (2017). Novel genetic loci associated with hippocampal volume. *Nat Commun* **8**:13624.

Higuchi F, Uchida S, Yamagata H, Abe-Higuchi N, Hobara T, Hara K, *et al.* (2016). Hippocampal microRNA-124 enhances chronic stress resilience in mice. *J Neurosci* **36**:7253–7267.

Hobara T, Uchida S, Otsuki K, Matsubara T, Funato H, Matsuo K, *et al.* (2010). Altered gene expression of histone deacetylases in mood disorder patients. *J Psychiatr Res* **44**:263–270.

Inkster B, Nichols TE, Saemann PG, Auer DP, Holdboer F, Muglia P, Matthews PM (2009). Association of GSK3beta polymorphisms with brain structural changes in major depressive disorder. *Arch Gen Psychiatry* **66**:721–728.

Inkster B, Nichols TE, Saemann PG, Auer DP, Holsboer F, Muglia P, Matthews PM (2010). Pathway-based approaches to imaging genetics association studies: Wnt signaling, GSK3beta substrates and major depression. *Neuroimage* **53**:908–917.

Inkster B, Zai G, Lewis G, Miskowiak KM (2018). GSK3β: a plausible mechanism of cognitive and hippocampal changes induced by erythropoietin treatment in mood disorders? *Translational Psychiatry*. [In press].

Jope RS, Cheng Y, Lowell JA, Worthen RJ, Sitbon YH, Beurel E (2016). Stressed and Inflamed, Can GSK3 Be Blamed? *Trends Biochem Sci* **42**:180–192.

Kakiuchi C, Iwamoto K, Ishiwata M, Bundo M, Kasahara T, Kusumi I, *et al.* (2003). Impaired feedback regulation of XBP1 as a genetic risk factor for bipolar disorder. *Nat Genet* **35**:171–175.

Kakiuchi C, Ishiwata M, Nanko S, Kunugi H, Minabe Y, Nakamura K, *et al.* (2007). Association analysis of HSP90B1 with bipolar disorder. *J Hum Genet* **52**:794–803.

Kim WY, Snider WD (2011). Functions of GSK-3 signaling in development of the nervous system. *Front Mol Neurosci* **4**:44–47.

Kurauchi Y, Hisatsune A, Seki T, Katsuki H (2016). Na(+), K(+)-ATPase dysfunction causes cerebrovascular endothelial cell degeneration in rat prefrontal cortex slice cultures. *Brain Res* **1644**:249–257.

Lewis CM, Ng MY, Butler AW, Cohen-Woods S, Uher R, Pirlo K, *et al.* (2010). Genome-wide association study of major recurrent depression in the U.K. population. *Am J Psychiatry* **167**:949–957.

Li X, Jope RS (2010). Is glycogen synthase kinase-3 a central modulator in mood regulation? *Neuropsychopharmacology* **35**:2143–2154.

Linding R, Jensen LJ, Ostheimer GJ, van Vugt MA, Jørgensen C, Miron IM, *et al.* (2007). Systematic discovery of in vivo phosphorylation networks. *Cell* **129**:1415–1426.

Loh PR, Danecek P, Palamara PF, Fuchsberger C, Reshev YA, Finucane HK, *et al.* (2016). Reference-based phasing using the Haplotype Reference Consortium panel. *Nat Genet* **48**:1443–1448.

McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, *et al.* (2016). A reference panel of 64 976 haplotypes for genotype imputation. *Nat Genet* **48**:1279–1283.

Mechawar N, Savitz J (2016). Neuropathology of mood disorders: do we see the stigmata of inflammation? *Transl Psychiatry* **6**:e946.

Muglia P, Tozzi F, Galwey NW, Francks C, Upmanyu R, Kong XQ, *et al.* (2010). Genome-wide association study of recurrent major depressive disorder in two European case-control cohorts. *Mol Psychiatry* **15**:589–601.

Nevell L, Zhang K, Aiello AE, Koenen K, Galea S, Soliven R, *et al.* (2014). Elevated systemic expression of ER stress related genes is associated with stress-related mental disorders in the Detroit Neighborhood Health Study. *Psychoneuroendocrinology* **43**:62–70.

Nicodemus KK (2011). Letter to the editor: on the stability and ranking of predictors from random forest variable importance measures. *Brief Bioinform* **12**:369–373.

Nicodemus KK, Malley JD (2009). Predictor correlation impacts machine learning algorithms: implications for genomic studies. *Bioinformatics* **25**:1884–1890.

Nicodemus KK, Law AJ, Radulescu E, Luna A, Kolachana B, Vakkalanka R, *et al.* (2010a). Biological validation of increased schizophrenia risk with NRG1, ERBB4, and AKT1 epistasis via functional neuroimaging in healthy controls. *Arch Gen Psychiatry* **67**:991–1001.

Nicodemus KK, Callicott JH, Higier RG, Luna A, Nixon DC, Lipska BK, *et al.* (2010b). Evidence of statistical epistasis between DISC1, CIT and NDEL1 impacting risk for schizophrenia: biological validation with functional neuroimaging. *Hum Genet* **127**:441–452.

Nicodemus KK, Malley JD, Strobl C, Ziegler A (2010c). The behaviour of random forest permutation-based variable importance measures under predictor correlation. *BMC Bioinformatics* **11**:110–112.

O'Leary O, Nolan Y (2015). Glycogen synthase kinase-3 as a therapeutic target for cognitive dysfunction in neuropsychiatric disorders. *CNS Drugs* **29**:1–15.

Pardo M, Abrial E, Jope RS, Beurel E (2016). GSK3β isoform-selective regulation of depression, memory and hippocampal cell proliferation. *Genes Brain Behav* **15**:348–355.

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D (2006). Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* **38**:904–909.

Ramasamy A, Trabzuni D, Guelfi S, Varghese V, Smith C, Walker R, *et al.* (2014). Genetic variability in the regulation of gene expression in ten regions of the human brain. *Nat Neurosci* **17**:1418–1428.

Roy B, Dunbar M, Shelton RC, Dwivedi Y (2017). Identification of microRNA-124-3p as a putative epigenetic signature of major depressive disorder. *Neuropsychopharmacology* **42**:864–875.

Rubovitch V, Barak S, Rachmany L, Goldstein RB, Zilberstein Y, Pick CG (2015). The neuroprotective effect of salubrinal in a mouse model of traumatic brain injury. *Neuromolecular Med* **17**:58–70.

Sarkar A, Chachra P, Kennedy P, Pena CJ, Desouza LA, Nestler EJ, *et al.* (2014). Hippocampal HDAC4 contributes to postnatal fluoxetine-evoked depression-like behavior. *Neuropsychopharmacology* **39**:2221–2232.

Schmaal L, Veltman DJ, van Erp TG, Sämann PG, Frodl T, Jahanshad N, *et al.* (2016). Subcortical brain alterations in major depressive disorder: findings from the ENIGMA Major Depressive Disorder working group. *Mol Psychiatry* **21**:806–812.

Sharma S, Taliyan R (2015). Synergistic effects of GSK-3β and HDAC inhibitors in intracerebroventricular streptozotocin-induced cognitive deficits in rats. *Naunyn Schmiedebergs Arch Pharmacol* **388**:337–349.

Strekalova T, Markova N, Shevtsova E, Zubareva O, Bakhmet A, Steinbusch HM, *et al.* (2016). Individual differences in behavioural despair predict brain GSK-3beta expression in mice: the power of a modified swim test. *Neural Plast* **2016**:5098591.

Timberlake MA 2nd, Dwivedi Y (2016). Altered expression of endoplasmic reticulum stress associated genes in hippocampus of learned helpless rats: relevance to depression pathophysiology. *Front Pharmacol* **6**:319–321.

Tozzi F, Prokopenko I, Perry JD, Kennedy JL, McCarthy AD, Holsboer F, *et al.* (2008). Family history of depression is associated with younger age of onset in patients with recurrent depression. *Psychol Med* **38**:641–649.

Uher R, Farmer A, Maier W, Rietschel M, Hauser J, Marusic A, *et al.* (2008). Measuring depression: comparison and integration of three scales in the GENDEP study. *Psychological Medicine* **38**:289–300.

Wing JK, Babor T, Brugha T, Burke J, Cooper JE, Giel R, *et al.* (1990). SCAN. Schedules for Clinical Assessment in Neuropsychiatry. *Arch Gen Psychiatry* **47**:589–593.

Wu Y, Hou F, Wang X, Kong Q, Han X, Bai B (2016). Aberrant expression of histone deacetylases 4 in cognitive disorders: molecular mechanisms and a potential target. *Front Mol Neurosci* **9**:114–117.

Yamada N, Makino Y, Clark RA, Pearson DW, Mattei MG, Guénet JL, *et al.* (1994). Human inositol 1,4,5-trisphosphate type-1 receptor, InsP3R1: structure, function, regulation of expression and chromosomal localization. *Biochem J* **302**:781–790.

Yang Y, Qin X, Liu S, Li J, Zhu X, Gao T, Wang X (2011). Peroxisome proliferator-activated receptor γ is inhibited by histone deacetylase 4 in cortical neurons under oxidative stress. *J Neurochem* **118**:429–439.

Zhang Y, Liu W, Zhou Y, Ma C, Li S, Cong B (2014). Endoplasmic reticulum stress is involved in restraint stress-induced hippocampal apoptosis and cognitive impairments in rats. *Physiol Behav* **131**:41–48.

Zhao Y, Chen F, Zhai R, Lin X, Wang Z, Su L, *et al.* (2012). Correction for population stratification in random forest analysis. *Int J Epidemiol* **41**:1798–1806.