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Gene expression in peripheral blood in treatment-free major depression

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Original Article

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Abstract

Background: Peripheral gene expression of several molecular pathways has been studied in major depressive disorder (MDD) with promising results. We sought to investigate some of these genes in a treatment-free Latino sample of Mexican descent. *Material and Methods:* The sample consisted of 50 MDD treatment-free cases and 50 sex and age-matched controls. Gene expression of candidate genes of neuroplasticity (*BDNF, p11,* and *VGF*), inflammation (*IL1A, IL1B, IL4, IL6, IL7, IL8, IL10, MIF,* and *TNFA*), the canonical Wnt signaling pathway (*TCF7L2, APC,* and *GSK3B*), and mTOR, was compared in cases and controls. RNA was obtained from blood samples. We used bivariate analyses to compare subjects versus control mean mRNA quantification of target genes and lineal regression modelling to test for effects of age and body mass index on gene expression. *Results:* Most subjects were female (66%) with a mean age of 26.7 (SD 7.9) years. Only *GSK3B* was differentially expressed between cases and controls at a statistically significant level ($p = 0.048$). *TCF7L-2* showed the highest number of correlations with MDD-related traits, yet these were modest in size. *Discussion: GSK3B* encodes a moderator of the canonical Wnt signaling pathway. It has a role in neuroplasticity, neuroprotection, depression, and other psychiatric phenotypes. We found that adding population diversity has the potential to elicit distinct peripheral gene expression markers in MDD and MDD-related traits. However, our results should only be considered as hypothesis-generating research that merits further replication in larger cohorts of similar ancestry.

Significant outcomes

- *GSK3B* shows a positive association with treatment-free MDD Latino cases.
- Other pathways of interest such as inflammation and neuroplasticity did not differ significantly between MDD cases and controls.
- *TCF7L2* shows the highest number of correlations – of modest size – with MDD-related traits, such as self-esteem, vulnerable cognitive styles, and cognitive complaints.
- To our knowledge, this is the first study of gene expression in major depression in a Latino sample.

Limitations

- Peripheral expression does not necessarily reflect changes in brain or brain function.
- Sample size is small, and the lack of differences may be due to low statistical power.
- Candidate gene studies may prompt false negatives or false positive results, thus independent replication is needed to confirm findings.
- The dearth of similar studies in Latino samples hinder our capacity to compare our results with previous literature.
- The significant *p*-value is not adjusted for multiple comparisons and should be considered as hypothesis generation information.

Introduction

Major depressive disorder (MDD) has an important impact in loss of health and function (Ustün *et al.*, 2004), ranking as the main contributor to global disability (Whiteford *et al.*,

2013). The World Health Organization reported an estimate of 4.4% of the global population living with MDD – 5.1% of women and 3.6% of men – and a disquieting increase of 18% from 2005 to 2015 (World Health Organization, 2017). Moreover, the clinical presentation of MDD is heterogeneous and complex (Goldberg, 2011), with varying degrees of response to treatment (Petersen *et al.*, 2005) and important comorbidities (Kessler *et al.*, 2003). Nevertheless, diagnostic tools (DSM-5 and ICD10) and clinical management guides for MDD (NICE and CANMAT) still rely on symptomatology and lack the integration of biomarkers and precision medicine data (Perlis, 2016).

Approximately 40% of MDD liability is attributable to genetic factors (Kendler *et al.*, 2006), and although there is an important road ahead in determining the genetic variants responsible for this risk, recent studies have made important advances in finding them, since described samples already include more than 100 000 subjects (Wray *et al.*, 2018). On the other hand, early MDD gene expression studies showed that baseline differences between cases and controls inflammatory gene expression were associated with antidepressant treatment response (Cattaneo *et al.*, 2013). These promising results have led to the exploration of gene expression in MDD as baseline and treatment response markers.

Multiple physiological systems have been investigated as potential sources of biomarkers for MDD, including inflammatory and neurotrophic systems (Jani *et al.*, 2015; Strawbridge, Young and Cleare, 2017). One of the most representative research areas in this field is the neuroimmunological dysfunction hypothesis of depression (Miller, Maletic and Raison, 2009), in which major depression is the clinical end point of chronic stressors that modify stress response systems, which subsequently increase pro-inflammatory cytokines (Slavich and Irwin, 2014). Immunological gene expression studies, ascertaining the differences in the inflammatory system between subjects with depression and non-depressed controls, have revealed a higher expression of pro-inflammatory cytokines (IL-1β, IL-6, IL10, IFN-γ, and TNF-α), and a lower expression of IL-4 – an anti-inflammatory cytokine – in people with depression (Tsao *et al.*, 2006; Belzeaux *et al.*, 2010; Cattaneo *et al.*, 2013; Jansen *et al.*, 2016).

Another important hypothesis is that of synaptic plasticity disruption, supported by research suggesting that alterations of the mechanisms in charge of synaptic plasticity maintenance and control result in a destabilisation of cerebral networks related to mood and emotions (Duman and Aghajanian, 2012). This is not surprising, as neurotrophins (members of the nerve growth factor family) play a major role in brain development and plasticity of the mature central nervous system (Thoenen, 1995). There is evidence of disruption of regular neuroplasticity in major depression (Pittenger and Duman, 2008; Player *et al.*, 2013; Noda *et al.*, 2018), and studies suggest that Brain-derived neurotrophic factor (BDNF) is lower in the serum of treatment-free depressed subjects and normalises after treatment with antidepressants (Molendijk *et al.*, 2014). Furthermore, genetic studies have replicated this finding with mRNA data (Cattaneo *et al.*, 2010, 2013), as well as a post-mortem study that reported increased expression of BDNF in the brains of people previously treated with antidepressants (Chen *et al.*, 2001).

The canonical Wnt signaling pathway has garnered data that make it a promising system to explore in mood disorder research as well (Duman and Voleti, 2012). In psychiatry, evidence has accumulated on the participation on the Wnt signaling pathway in several diseases and related phenotypes (Mulligan and Cheyette, 2017) such as schizophrenia, (Miyaoka, Seno and Ishino, 1999; Levchenko *et al.*, 2015; Hoseth *et al.*, 2018), bipolar disorder (BD) (Matigian *et al.*, 2007; Zandi *et al.*, 2008; Sani *et al.*, 2012; Winham *et al.*, 2014; Pandey *et al.*, 2015; Cuellar-Barboza *et al.*, 2016; Hoseth *et al.*, 2018), anxiety disorders (Zhao *et al.*, 2018; Vidal *et al.*, 2019), and depression (Saus *et al.*, 2010; Enatescu *et al.*, 2016; Peng *et al.*, 2018; Vidal *et al.*, 2019). Several members of the Wnt signaling pathway have been specifically linked to mood disorders.

Although there has been a steady increase in the amount of research dedicated to the study of these three hypotheses of depression and their interconnectedness, efforts to replicate these findings in distinct populations are lacking (Frodl, 2017). This is a priority since population diversity is an important source of genetic heterogeneity (Hodgson, McGuffin and Lewis, 2017) and a major limitation in the generation of both diagnostic and treatment response genetic biomarkers of depression (Mora *et al.*, 2018).

Aims of the study

In this cross-sectional study of a Latino sample, we sought to investigate gene expression markers of MDD and MDD-related traits, by comparing peripheral gene expression levels between depressed treatment-free subjects and mentally healthy controls. We evaluated 16 genes, key members of 3 different biological pathways, namely: *IL1A, IL1B, IL4, IL6, IL7, IL8, IL10, MIF,* and *TNFA* (inflammatory pathway); *BDNF, p11* and *VGF* (synaptic plasticity and neurotrophism pathway); *TCF7L2*, APC, and *GSK3B* (canonical Wnt signaling pathway), and *mTOR*.

Materials and methods

Subjects

This project was carried out at the Affective Health Center of the Department of Psychiatry of the University Hospital, Universidad Autónoma de Nuevo Leon, Monterrey, Mexico. It was part of a larger naturalistic study designed to measure peripheral gene expression at baseline and after antidepressant treatment with a selective serotonin reuptake inhibitor treatment in adults with an MDD diagnosis, without treatment at recruitment. The study protocol and procedures were approved by the Institutional Review Board[1], and written informed consent was obtained from all subjects prior to participation. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. This study is also registered under Project No. 272616 of the Sectoral Fund of Research in Health and Social Security SS/IMSS/ISSSTE of the National Council of Science and Technology (CONACYT).

After screening with the Patient Health Questionnaire-9 > 5 (Kroenke, Spitzer and Williams, 2001), subjects aged 18 to 65 years with a diagnosis of MDD (single episode or recurrent; moderate to severe), and at least treatment-free for 6 months, were included. Candidates who fulfilled diagnostic criteria for psychotic, bipolar I or II, obsessive-compulsive, or severe alcohol use disorders were not eligible for participation. Diagnoses were performed by a board-certified psychiatrist according to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV-TR) criteria

[1]Procedures and protocols were approved by the Research Ethics Committee on September 14, 2016 (No. PS16-00028).

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using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First *et al.*, 2007). Body mass index (BMI) was calculated as the weight of the subject divided by the square of the body height and expressed as kg/m^2. Waist circumference was measured at the midpoint between the last palpable rib and the iliac crest.

Montgomery–Asberg Depression Rating Scale (MADRS) scores were used as a measure of symptom severity (Montgomery and Asberg, 1979; Snaith *et al.*, 1986). Self-report additional clinical scales used in the group of cases were Generalized Anxiety Disorder 7 (Spitzer *et al.*, 2006), Pittsburgh Sleep Quality Index (Buysse *et al.*, 1989), Rosenberg Self-Esteem Scale (RSES) (Rosenberg, 1965), British Columbia Cognitive Complaints Inventory (BC-CCI) (Iverson and Lam, 2013), and Cognitive Styles Questionnaire Short Form (CSQ-SF) (Meins *et al.*, 2012).

Our study enrolled 100 people in 2 groups: treatment-free depressed cases ($n = 50$) and mentally healthy controls ($n = 50$). Sex- and age-matched (± 1 year) mentally healthy controls were administered the SCID-I, the MADRS, and the Patient Health Questionnaire. Individuals were included in the control group when they had no lifetime mood or anxiety disorders and a low score (≤ 6) on the MADRS.

RNA extraction and cDNA synthesis

We collected 4 mL of peripheral blood from cases with MDD and mentally healthy controls into anticoagulant-prepared tubes (EDTA). Total RNA was extracted using the NucleoSpin® RNA Blood kit (MACHEREY-NAGEL GmbH & Co. KG). The quality of the RNA was determined by Bioanalyzer (Agilent Technologies). Complementary RNA (cDNA) was synthesised from 1 µg of RNA with the SuperScript IV Vilo Master Mix kit (ThermoFisher) in accordance with the manufacturer's instructions.

Gene expression analysis

Gene expression was measured through quantitative PCR (qPCR), using the Step One Plus detection system (Applied Biosystems™) in a 96-well plate format. As internal controls, three genes were used: Beta-2-Microglobulin (*B2M*), Glyceraldehyde 3-Phosphate Dehydrogenase (*GAPDH*), and Ribosomal Protein Lateral Stalk Subunit P0 (*RPLPO*). These were also used for data normalisation.

The TaqMan probes for each gene of interest (*IL1A, IL1B, IL4, IL6, IL7, IL8, IL10, MIF, TNFA, BDNF, p11, VGF, TCF7L2, APC, mTOR,* and *GSK3B*) were obtained from Applied Biosystems. The qPCR proceeded as follows: 1 cycle at 95 ° C for 5 min to activate the polymerase, 50 cycles were performed; each cycle consisted of a step of denaturation at 95 ° C for 30 s, an alignment step at 60 ° C for 30 s and an elongation step at 72 ° C for 30 s.

The values of Ct were normalised with the software ArrayStudio (Qiagen). Relative quantification values of mRNA were obtained using the $2^{-\Delta\Delta Ct}$ comparison method. Amplification reactions were performed in triplicate with determined reproducibility.

Statistical analysis

Parametric (Student's *t*-test for BMI) and nonparametric (Mann–Whitney *U*-test for waist) were used to test the differences between the groups depending on normality of the sampled values. Given the challenges of accurately determining the distribution of a qPCR gene expression sample, due to dispersion not being well represented in scenarios with a limited number of biological replicates available, a normal distribution was assumed and parametric tests for gene expression differences (Goni, García and Foissac, 2009) were performed, including an ANOVA [ArrayStudio (Qiagen)].

Linear regression models with age, gender, and BMI as covariates were developed for each gene. Also, correlations between gene expression and clinical traits of depression, anxiety, self-esteem, cognitive styles and cognitive vulnerability were tested (Spearman's rho test). A $p < 0.05$ value was considered statistically significant.

Results

Our study enrolled 50 treatment-free depressed subjects and 50 mentally healthy controls. Sixty-six per cent of the total sample were women, with a mean age of 26.7 years (SD 7.9). No significant differences were found between the groups in BMI ($t = 1.81$, $p = 0.07$) or waist circumference ($U = 1145.50$, $p = 0.97$). Cases had a mean MADRS score of 31.9 (SD 7.8); among them, 68% had moderate depression and 32% had severe depression. Sixty-two per cent of the cases had no history of a previous depressive episode. The demographic and clinical data of subjects are presented in Table 1.

Case and control-grouped gene expression levels are shown in Figure 1. No group-level differences were found between genes belonging to the inflammation or neurotrophic pathways, while a statistically significant difference was found for *GSK3B* ($p = 0.048$), a member of the canonical Wnt signaling pathway.

Linear regression modelling did not show an influence of age, gender or BMI on gene expression levels, except for a small effect of age on *IL1A* ($R^2 = 0.081$, $p = 0.017$) and *IL6* ($R^2 = 0.107$, $p = 0.001$) expression. *TCF7L2* ($r = 0.297$, $p = 0.036$) and *IL6* ($r = 0.421$, $p = 0.004$) showed a positive correlation with self-esteem as measured by the RSES. *TCF7L2* showed a negative correlation with the total vulnerability score of the CSQ-SF ($r = -0.296$, $p = 0.037$). *IL8* ($r = 0.355$, $p = 0.011$) had a positive correlation with cognitive complaints assessed by the BC-CCI, while *MIF* ($r = -0.295$, $p = 0.04$) and *TCF7L2* ($r = -0.284$, $p = 0.045$) showed negative correlations with the same scale. Complete correlation results are shown in Supplementary Table 1.

Discussion

In this study, we tested the blood gene expression of 16 genes from 3 different biological pathways, namely key members of inflammation, synaptic plasticity and neurotrophism pathways, and the canonical Wnt signaling pathway. We compared 50 treatment-free depressed cases with 50 age and sex-matched mentally healthy controls of Mexican descent and found a statistically significant association of higher *GSK3B* expression levels in cases versus controls. Additionally, when testing for correlations between gene expression and traits associated with MDD, *TCF7L2* showed the highest number of significant correlations, positive with self-esteem, and negative with cognitive vulnerability and cognitive complaints – yet they were of modest size and the *p*-values are only normal since they were not corrected for multiple comparisons. To our knowledge, this study is not only the first Latino population study of gene expression in major depression but also the first study to explore Wnt signaling members as candidate gene expression biomarkers of MDD. Finally, and again to our knowledge, as the first study to explore gene expression correlations between gene expression levels and cognitive traits related to MDD, it provides interesting hypothesis-generating results.

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Table 1. Sociodemographic and clinical data of our study sample ($n = 100$)

	Cases ($n = 50$)	Controls ($n = 50$)
Age	26.74 (SD 8.1)	26.62 (SD 7.9)
Sex		
Female	33 (66.0%)	33 (66.0%)
Male	17 (34.0%)	17 (34.0%)
Anthropometry		
BMI (kg/m^2)	25.68 (SD 5.6)	23.98 (SD 3.5)
Waist (cm)	82.11 (SD 15.2)	80.39 (SD 10.9)
Marital status		
Single	37 (74.0%)	41 (82.0%)
Married	6 (12.0%)	8 (16.0%)
Other	7 (14.0%)	1 (2.0%)
Academic achievement		
Jr. High	1 (2.0%)	0 (0.0%)
High school	34 (68.0%)	31 (62.0%)
Graduate	13 (26.0%)	13 (26.0%)
Postgraduate	2 (4.0%)	6 (12.0%)
Clinical scales		
MADRS	31.90 (SD 7.8)	1.38 (SD 1.8)
GAD-7	13.80 (SD 5.0)	-
PSQI	10.96 (SD 3.4)	-
RSES	23.14 (SD 5.3)	-
BC-CCI	14.68 (SD 4.9)	-
CSQ-SF	224.56 (SD 41.4)	-
Depressive disorder subtype		
Single episode	31 (62.0%)	-
Recurrent	19 (38.0%)	-
Follow-up outcomes		
Response	32 (62.0%)	-
Remission	21 (42.0%)	-

Note. No significant differences were found between case and control BMI ($t = 1.81$, $p = 0.07$) or waist circumference ($U = 1145.50$, $p = 0.97$).
BMI, body mass index; MADRS, Montgomery–Asberg Depression Rating Scale; GAD-7, Generalized Anxiety Disorder 7; RSES, Rosenberg Self-Esteem Scale; BC-CCI, British Columbia Cognitive Complaints Inventory; CSQ-SF, Cognitive Styles Questionnaire Short Form.

The canonical Wnt signaling pathway has a key role in the regulation of neurogenesis and synaptic plasticity; moreover, several members of this pathway have been involved in mood disorders and their treatment (Inkster *et al.*, 2018).Glycogen synthase kinase-3 beta (GSK3B) is an inactivator of the canonical Wnt signaling pathway and also has a role in other pathways related to neuronal development and function. It is a multifunctional serine/threonine protein kinase that contributes to diverse cell functions, including gene expression, neurogenesis, neuroplasticity, cell survival, differentiation, migration, stress responses, and apoptosis, in the immune system, neurotransmitter systems, metabolism, and other functions (Hur and Zhou, 2010; Valvezan and Klein, 2012). GSK-3B has been found to mediate depressive symptoms in a chronic stress mouse model (Peng *et al.*, 2018), and its inhibition has been shown to produce antidepressant-like effects in other animal models of depression (Gould *et al.*, 2004; Kaidanovich-Beilin *et al.*, 2004). Variation in grey matter volume in the hippocampus and superior temporal gyrus of depressed subjects has been associated with *GSK3B* polymorphisms (Inkster *et al.*, 2009), and, in a post-mortem study of 20 depressed and 20 non-depressed subjects, Karege and collaborators found significant differences in protein levels of GSK-3B and β-catenin (Karege *et al.*, 2012). A similar study in post-mortem brains of BD and schizophrenia subjects showed statistically significant differences in protein and gene expression levels of *GSK3B* and β-catenin in bipolar versus controls, but not in the schizophrenia group (Pandey *et al.*, 2015), suggesting a potential role in mood disorders. Moreover, BD and MDD can be treated with lithium, an agent that inhibits GSK3B as part of its mechanisms of action (Manji, Moore and Chen, 2000; Valvezan and Klein, 2012). Our findings are consistent with this thread, providing early evidence of differential gene expression of *GSK3B* in MDD.

TCF7L2 on the other hand encodes a transcription factor of the same name, downstream from the canonical Wnt signaling pathway (Saito-Diaz *et al.*, 2013). Variants in this gene have been associated with type 2 diabetes risk (Manning *et al.*, 2012) and to BD when accounting for obesity (Winham *et al.*, 2014; Cuellar-Barboza *et al.*, 2016). Associations of the expression of this gene and BMI or waist were not found in our sample, perhaps due to insufficient statistical power (Winham and Biernacka, 2013). However, we found that *TCF7L2* gene expression had the highest number of correlations with MDD-related traits, namely, it was positively correlated with self-esteem and negatively correlated with cognitive traits associated with MDD vulnerability (Chu *et al.*, 2017; Mac Giollabhui *et al.*, 2018), which lends support to its biological role in neuroplasticity, neurogenesis, and brain patterning, among others (Kim and Snider, 2011). Research on the canonical Wnt pathway in psychiatric conditions such as BD has shown that cellular lines derived from induced pluripotent stem cells from cases differ importantly in this pathway from those derived from mentally healthy controls (Madison *et al.*, 2015). However, its role in MDD has not been explored to the same extent, and our results support further investigation in this disorder. Our exploratory, hypothesis-generating analyses of MDD-related clinical measures and gene expression, reports parameters for the population our sample came from, Latinos of Mexican descent, and supports the merit of future longitudinal studies to establish directionality and gene–environmental interactions that may elucidate whether these cognitive traits predispose cells to an abnormal gene expression (or vice versa) and the mediator environmental factors that may contribute to this effect.

Despite the evidence supporting the role of inflammatory pathways as molecular pathways involved in MDD, conflicting evidence on the role of baseline gene expression of these markers in MDD has emerged (Tsao *et al.*, 2006; Wright *et al.*, 2014). Our study did not find differential expression of members of these pathways in MDD cases versus controls, a finding that may be due to a different ancestry from the European Americans recruited in previous investigations, to the limitation of candidate gene studies (Duncan, Ostacher and Ballon, 2019), or to other factors. Jansen et al., for instance, did not find differential expression at baseline per se; however, when exploring these markers at the pathway level, they found positive associations for inflammation (Jansen *et al.*, 2016). Our limited number of investigated genes and our sample size prevent from attempting this sort of analysis.

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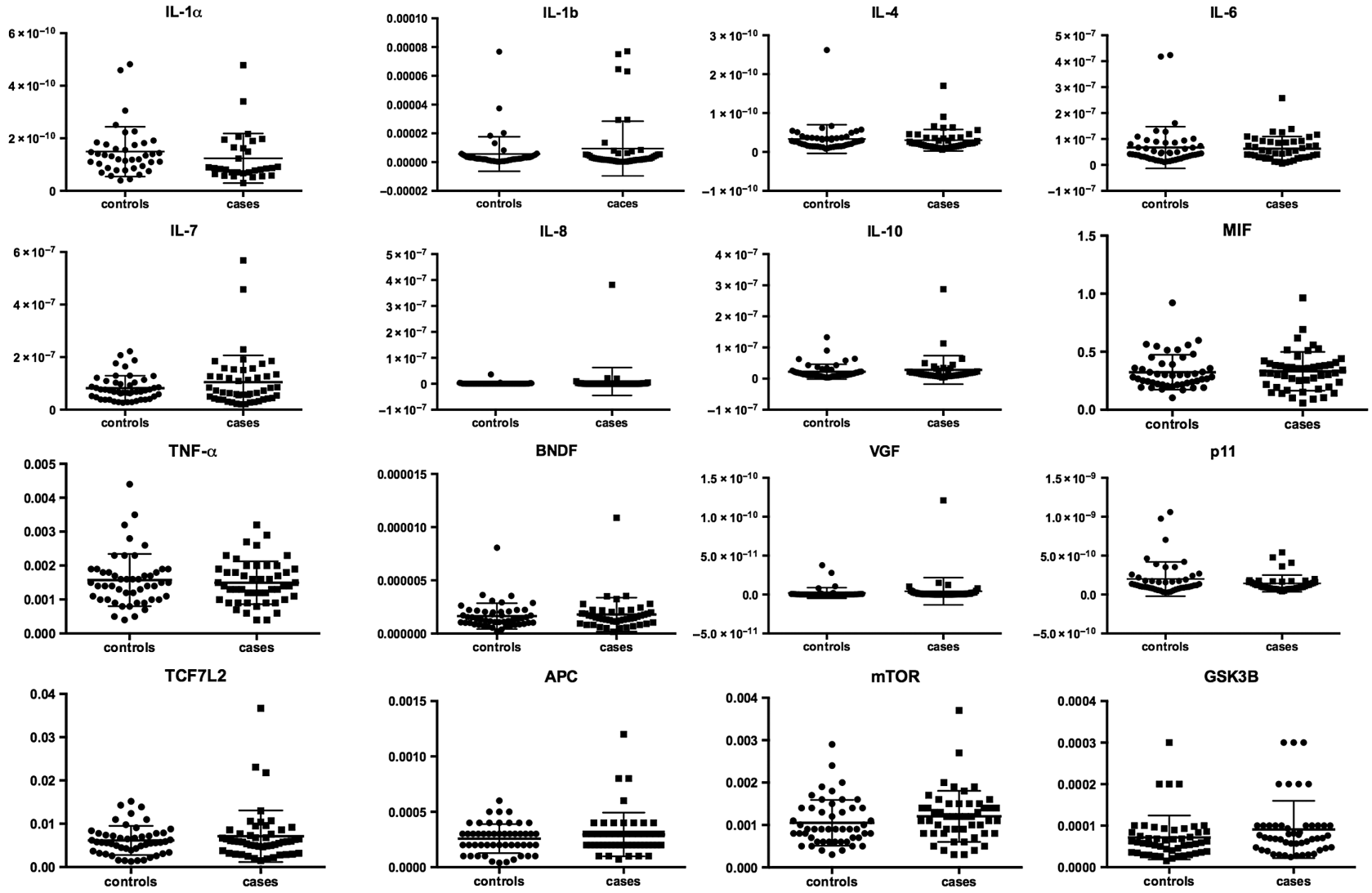

Fig. 1. Gene expression of all subjects by group. All 16 genes are accommodated according to their biological pathway (inflammation, neurotrophism, and canonical Wnt signaling).
Note: no significant differences were found between case and control gene expression for most genes, except GSK3B ($p = 0.0483$). NS, non-significance; *$p < 0.05$.

There are several limitations to our study. First, its cross-sectional design hinders our ability to understand the directionality of its results. Second, we used whole blood analysis of gene expression, and several of the genes in this study (such as *GSK3B*) are differentially expressed in whole blood (median Transcripts Per Million (TPM) = 9.340) when compared to other tissues and organs, and these determinations are not necessarily correlated with their level of expression in the brain (Ciobanu *et al.*, 2016). Specifically, certain brain tissues have a particularly high median TPM values, including the cerebellar hemispheres (median TPM = 37.42), the frontal cortex (median TPM = 25.49), and the hypothalamus (median TPM = 17.17) (Data Source: GTEx Analysis Release V7). Even though the statistical analysis of group-level gene expression differences shows a nominally significant difference for one of the genes analysed, we must be cautious when interpreting these data since no multiple testing corrections were made. Testing for the expression of these genes in other tissues and a larger sample size seems warranted. Finally, the dearth of similar studies in Latino populations hinder our capacity to compare our results with previous literature.

It is also important that there is noticeable variability in the methodology for the quantification of gene expression in studies of psychiatric samples published over the last 2 years. When considering only studies that reported their method of gene expression quantification, the most commonly used method was the comparative $2^{-\Delta\Delta Ct}$ method, but there were variations in the data normalisation process: use of one reference gene (Amidfar *et al.*, 2017; Bobińska, Gałecka, *et al.*, 2017; Bobińska, Mossakowska-Wójcik, *et al.*, 2017; Liu *et al.*, 2017; Yang *et al.*, 2017; Fries *et al.*, 2017; Gałecka *et al.*, 2017, 2018; Ghafelehbashi *et al.*, 2017; Hoseth *et al.*, 2017; Hung *et al.*, 2017; Akcan *et al.*, 2018; Sao *et al.*, 2018), relativity to their own endogenous control (Doolin *et al.*, 2017), two reference genes (Roy *et al.*, 2017), and geometric mean of two reference genes (Chau *et al.*, 2018). This source of variability may difficult the interpretation (and comparison) of the results of gene expression studies of psychiatric conditions; moreover, it impedes the realisation of meta-analysis.

Important strengths of this study include the originality of the selected pathways. The inclusion of cases that had been treatment-free for at least 6 months – which is substantially larger than most gene expression studies of MDD – therefore, avoiding the potentially confounding effect of antidepressants, in addition to a clinical phenotype rigorously evaluated by a board-certified psychiatrist with expertise in mood disorders, help to avoid important sources of heterogeneity (Kendler *et al.*, 2013). Another contribution lies in the fact that it is the first exploration of the relationship between cognitive MDD-related traits and gene expression. Finally, the inclusion of Latino subjects adds ancestry diversity to existing studies in the field, fomenting one of the genetics' current priorities (Hindorff *et al.*, 2018).

As the validation of biomarkers increases in consistency, calibration, and ability to discriminate between disease and treatment states, the confidence in diagnostic tools derived from them will also increase (Perlis, 2011), and as such the possibility to develop personalised treatment and finally, primary and secondary prevention strategies. It will be vital to avoid bias and overestimation through a more rigorous systematisation of future studies

Authors contributions

ABCB directed the project. ABCB, IPRS, LEM, and MIR participated in the conception and design of the study. ABCB, JASR, and SG conducted candidate interviews and ABCB conducted structured clinical interviews. GC, JL, and MIR performed the gene expression analysis from the samples. ABCB and JASR performed follow-up of subjects. JASR and SG performed database upkeep. ABCB, JASR, IPRS, SG, GC, JL, and MIR conducted analysis of data and participated in monthly meetings to discuss preliminary results. ABCB, JASR, SG, and MIR drafted the manuscript with contributions from GC, ACE, and JL. IPRS and ACE provided critical feedback and helped to shape the manuscript.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/neu.2020.5

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Statement of interest. None.

Conflicts of interest. None.

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